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A STUDY OF THE INTERACTIVE EFFECTS OF STRESSES FROM RESPIRATOR WEAR AND SIMULTANEOUS EXPOSURE TO TOXIC ANTICHOLINESTERASE AGENTS.

Final Comprehensive Report.

Walter Ehrlich, M.D.
CPT. Harry J. Quebbeman, MPH, PH.D. Candidate

June, 1983

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The cardiopulmonary effects of a peripheral cholinesterase inhibitor (0.025 mg neostigmine i.v. per kg) and inspriatory resistance loading comparable to the inspiratory resistance of a protective respirator mask, were studied comprehesively in 6 intact, awake dogs at rest and during exercise. The aim of this study was to test the hypothesis that both exercise and inspiratory resistance loading enhance the toxic effect of sublethal doses of CHEI agents. This study was also carried out in order to establish the methodological and physiological foundations for further in depth analysis of the pathophysiological and

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compensatory mechanism involved, and to explore possible and therapeutic interventions in the treatment of CHEI-exposed subjects.

We found that inspiratory resistance loading alone decreased tidal volume, minute volume, and inspiratory peak flow. Inspiratory muscle work was increased and became less efficient in producing ventilation. Afterload to the ejection of the left ventricle was elevated, which in turn increased the external work of the left heart. Oxygen consumption was slightly decreased in spite of enhanced respiratory work. Venous carbon dioxide pressure was elevated, commensurate with the increased muscular work of breathing.

Neostigmine inhibited whole blood cholinesterase activity by 50% and increased respiratory frequency, probably by cholinergic reflex. Minute volume and peak inspiratory air flow were increased at rest. During exercise these functions were decreased compared to control exercise values. Inspiratory muscle work was increased and became less efficient. Neostigmine diminished heart rate and cardiac output. Left ventricular afterload was diminished, and transmural pulmonary arterial pressure and transmural left and right atrial pressures were elevated. As a result, calculated left ventricular work rate was decreased. Blood volume was shifted from the periphery to the capacitance areas of the heart and lungs, contributing to a diminished systemic perfusion pressure and a falling pulmonary compliance. Arterial and venous oxygen pressures fell. Oxygen consumption at rest was not increased; during exercise it was diminished in spite of the demands of a large increase in respiratory work rate. Venous carbon dioxide pressure was elevated, while venous and arterial pH fell. Gas exchange in the lungs was compromised, as evidenced by a calculated increase in shunt fraction. The increase of inspiratory muscle work caused by each of the three challenges (neostigmine injection, inspiratory resistance loading, and exercise) were mutually additive, and as inspiratory resistance loading, to exercise, or to both challenges together, always caused a fall in cardiac output and in arterial pressure, suggesting a failure of the heart to respond to the increased demands of the respiratory muscles. This non-homeostatic condition was further aggravated by compromised pulmonary gas exchange and acidosis secondary to the cholinesterase inhibition.

Conclusions: Mild peripheral cholinesterase inhibition endangers the mammal organism mainly by the fact that the increased metabolic needs of the respiratory muscles are not met as a result of diminished blood flow and perfusion pressure, and compromised gas exchange in the lung. The mechanical resistance load of a protective respirator, and the increased work of breathing associated with exercise, serve to aggravate these conditions to an extent that may lead to incipient respiratory muscle fatigue, and ultimately to peripheral respiratory failure if these stress conditions would be allowed (or required) to persist.

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Summary

The cardiopulmonary effects of a peripheral cholinesterase inhibitor (0.025 mg neostigmine i.v. per kg) and inspiratory resistance loading comparable to the inspiratory resistance of a protective respirator mask, were studied comprehensively in 6 intact, awake dogs at rest and during exercise. The aim of the study was to test the hypothesis that both exercise and inspiratory resistance loading enhance the toxic effect of sublethal doses of CHEI agents. This study was also carried out in order to establish the methodological and physiological foundations for further in depth analysis of the pathophysiological and compensatory mechanisms involved, and to explore possible preventive and therapeutic interventions in the treatment of CHEI-exposed subjects.

We found that inspiratory resistance loading alone decreased tidal volume, minute volume, and inspiratory peak flow. Inspiratory muscle work was increased and became less efficient in producing ventilation. Afterload to the ejection of the left ventricle was elevated, which in turn increased the external work of the left heart. Oxygen consumption was slightly decreased in spite of enhanced respiratory work. Venous carbon dioxide pressure was elevated, commensurate with the increased muscular work of breathing.

Neostigmine inhibited whole blood cholinesterase activity by 50% and increased respiratory frequency, probably by cholinergic reflex. Minute volume and peak inspiratory air flow were increased at rest. During exercise these functions were decreased compared to control exercise values. Inspiratory muscle work was increased and became less efficient. Neostigmine diminished heart rate and cardiac output. Left ventricular afterload was diminished, and transmural pulmonary arterial pressure and transmural left and right atrial pressures were elevated. As a result, calculated left ventricular work rate was decreased. Blood volume was shifted from the periphery to the capacitance areas of the heart and lungs, contributing to a diminished systemic perfusion pressure and a falling pulmonary compliance. Arterial and venous oxygen pressures fell. Oxygen consumption at rest was not increased; during exercise it was diminished in spite of the demands of a large increase in respiratory work rate. Venous carbon dioxide pressure was elevated, while venous arterial pH fell. Gas exchange in the lungs was compromised, as evidenced by a calculated increase in shunt fraction. The increase of inspiratory muscle work caused by each of the three challenges (neostigmine injection, inspiratory resistance loading, and exercise) were mutually additive, and as inspiratory work incrementally rose by addition of the three challenges, the effectiveness of that work in driving ventilation fell. The addition of neostigmine to inspiratory resistance loading, to exercise, or to both challenges together, always caused a fall in cardiac output and in arterial pressure, suggesting a failure of the heart to respond to the increased demands of the respiratory muscles. This non-homeostatic condition was further aggravated by compromised pulmonary gas exchange and acidosis secondary to the cholinesterase inhibition.

Conclusions. Mild peripheral cholinesterase inhibition endangers the mammal organism mainly by the fact that the increased metabolic needs of the respiratory muscles are not met as a result of diminished blood flow and perfusion pressure, and compromised gas exchange in the lung. The mechanical resistance load of a protective respirator, and the increased work of breathing associated with exercise, serve to aggravate these conditions to an extent that may lead to incipient respiratory muscle fatigue, and ultimately to peripheral respiratory failure, if these stress conditions would be allowed (or required) to persist.

Foreword

In conducting ⠆ research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals", prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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CHAPTER I.   INTRODUCTION AND LITERATURE REVIEW

A.   Introduction

Control of human exposure to airborne toxic contamina-
tion of the work place environment is often managed through
the employment of respiratory protective masks (an estimated
11.6 million users in the U.S. in 1979; Leidel, 1982).
However, field and laboratory performance testing has re-
vealed an alarming range of effective protection factors
that vary immensely depending on variables of individual
respiratory design, face fit, physical activity of the user,
and the physico-chemical nature of the contaminant "pro-
tected" against (Leidel, 1982). Recent test results reveal
that the actual degree of protection afforded by respirator
use in actual work conditions may be far worse than manu-
facturer- and government-published protection factors would
suggest (Burgess, 1983). The important inference to be drawn
from these findings is that a respirator wearer in an exposure
setting is the recipient of a largely unquantifiable, but
certainly greater dose of his toxic environment than was pre-
viously estimated (Guyton, 1967; Pritchard, 1976).

A second significant limitation of respiratory protective
devices is the imposition of a physiological load on the user.
The nature of this loading has been reviewed by Raven et al
(1979) and separately by James (1977). Both authors conclude

that even though some psychological and subjective comfort perceptions are involved, the most important factors associated with physiological load are the inspiratory air flow resistance and the added dead space of the mask. The physiological consequences of these two design factors include the following:

(1) hypoventilation, with minute ventilation decreased, principally due to a reduction in respiratory frequency.

(2) inspiratory time increases with the resistance load, and peak inspiratory flow rate is diminished.

(3) ventilatory compensation usually includes changes in rate and depth of breathing but the relative contribution of each is quite variable, dependent on the amount of resistance and individual differences. Minimization of the added work of breathing is held to be the overriding mechanism regulating this balance (Milic Emili, 1970; Cain and Otis, 1949).

(4) increased arterial PCO_2 results when ventilatory compensation is complete.

(5) pulmonary compliance decreases at higher resistance loads.

(6) ventilation distribution changes in the lung may occur.

(7) external work of breathing is increased, and respiratory muscle efficiency is decreased (McGregor and Becklake, 1961).

(8) decreased exercise tolerance (Craig et al, 1970; Gee et al, 1968; Demedts et al, 1973).

(9) increased ventilatory drive, secondary to elevated levels of rebreathed CO_2 brought about by the mask's dead space.

(10) circulatory responses to inspiratory loading have been reported by various investigators but the differing experimental conditions and conflicting results make generalizations impossible.

With these two major limitations of respiratory protective devices in mind (i.e., unpredictable exposure level and mask-induced cardiopulmonary stress), a significant question arises concerning the integrated result of these two insults acting simultaneously on the respiratory wearer: Will the acute toxic manifestations (however subtle or profound) of the environmental contaminant interact with the superimposed cardiopulmonary load of the respiratory protective device itself? The answer to that question undoubtedly varies depending on the chemical agent in use. The nature of the physiological response to such a combined challenge comes into better focus as the toxicological characteristics of a given contaminant are defined.

If the chemical agent produces an airway irritant response (like SO_2, ozone, HCl, etc.), we might expect to observe an interaction with the ventilatory responses induced by mask wear. We might predict increased work of breathing, or even

complete intolerance of the subject to continue wearing his "protective" device.

If the chemical agent exposure interferes with central or peripheral nervous system controls of respiration or circulation (like the organic solvents and other hydrocarbons), we might predict disruption of the finely tuned reflex control mechanisms that regulate respiratory and circulatory responses to respirator mask loads.

If the chemical agent somehow interferes with gas exchange or oxygen carrying ability of the blood (like CO, cyanide, etc.), we might predict rapid onset of hypoxemia or anaerobic metabolism leading to respiratory muscular fatigue; or even complete failure, if added to the work load already imposed on ventilatory mechanics and gas exchange by the respirator mask.

The list could go on and on, and we might make some educated guesses about possible interactions of these agent challenges with respirator stress. But the lingering fact is that they would be just that . . . guesses.

There is a clear lack of available data to assess the potential for, much less to quantify the extent of, interaction or additivity of two such simultaneously-acting loads on integrated cardiopulmonary functions. With the goal of contributing to fill that void in our understanding of simultaneously-acting stresses on human physiology, the following literature review, experimental design, and dis-

cussion of results are presented.

B.   Literature Review

To maximize the potential for interactive or additive effects
of an environmental chemical stress acting simultaneously
with the purely mechanical stress of respirator mask re-
sistance, it is useful to select an environmental or indus-
trial contaminant for study whose low-dose toxic manifes-
tations effect as many as possible of the physiological
mechanisms that are known to be stressed by respiratory
protective masks.

With that criterion in mind a review is presented
outlining the cardiopulmonary effects of exposure to a
family of compounds known as the anticholinesterase agents
(anti-ChE's), which find widespread use in agriculture and
industry as insecticides and in clinical and research
medicine as pharmacologic agents. As will be brought out
fully in the following review of the literature, these
compounds can produce many alterations in cardiopulmonary
mechanics as well as autonomic reflex controls of ventila-
tion and circulation - the very mechanisms involved in the
compensatory responses to respirator mask resistance loading.

1.   The Anti-cholinesterase Agents

Chemicals that inhibit acetylcholinesterase (AChE) are
called anti-cholinesterase agents (anti-ChE). They cause

acetylcholine (ACh) to accumulate at cholinergic receptor sites by inactivating the hydrolytic activity of AChE in neural tissues. Thus, they are capable of producing effects equivalent to excessive stimulation of cholinergic receptors throughout the central and peripheral nervous system (Taylor, 1980). In view of the widespread distribution of cholinergic neurons and the resulting lack of specificity of response to generalized cholinergic stimulation, it is not surprising that the anti-ChE agents as a group have received more extensive application as toxic agents, in the form of domestic and agricultural insecticides and potential chemical warfare agents, than as therapeutic drugs, although the group has been widely employed as experimental cholinergic agonists to study neuro-physiological mechanisms.

Prior to World War II only the "reversible" anti-ChE agents were generally known, of which physostigmine (eserine) is the most noteworthy example. During the 1940's a new class of highly toxic chemicals, the organophosphates, was developed in Europe as agricultural insecticides (parathion, malathion). There extreme toxicity was due in part to their "irreversible" inactivation of AChE, thereby eliciting long-lasting cholinergic effects. The terms "reversible" and "irreversible" as applied to carbamylester and organophosphorous compounds reflect largely quantitative temporal differences, since their pharmacologic actions are qualitatively similar. Both classes of drugs react covalently with the esterase enzymes in

essentially the same manner as does acetylcholine
(Hobbiger, 1976). The effect of anti-ChE agents, then, is
due to the prevention of hydrolysis of ACh by AChE at
sites of cholinergic transmission and the resulting pro-
longed presence of this neurotransmitter in the synaptic
cleft. Both classes of drug, once bound to the enzyme, may
be hydrolyzed to a greater or lesser degree, and this
"deactivation" of the enzyme-inhibitor complex proceeds
at a rate that is agent-specific. Generally the carbamate
inhibitors (physostigmine, neostigmine) are relatively
easily hydrolyzed (within 3-4 hours; Wilson and Harrison,
1961) and thus the term "reversible" has been applied. The
organophosphorous inhibitors (parathion, soman, DFP) are
much more slowly hydrolyzed and the stability of the phos-
phorylated enzyme may be further enhanced by "aging", which
results from the loss of one of the alkyl groups (Aldridge,
1976). Hence, the return of AChE activity depends on the
synthesis of new enzyme which may take up to 30 days for
complete recovery to pre-inhibition levels (Rosenberry, 1975).

a) Physiological Effects of Lethal Doses of Anti-ChE Agents

The onset of symptomatology and lethal effects resulting
from massive doses of anti-ChE have been widely studied. Death
is usually caused by respiratory failure resulting from weak-
ness of the muscles of respiration, central depression of
respiratory drive, and airway obstruction by bronchial and
salivary secretions, bronchoconstriction and occasionally

laryngospasm (Holmes, 1953, a,b,; DeCondole, 1953; Stewart and Anderson, 1968; Adams et al, 1976). In overwhelming exposure, when respiration fails rapidly, the circulation is relatively unimpaired until the point of death (Oberst, 1956). When exposure is less acute and death delayed, early circulatory failure, due at least in part to anoxia, complicates the respiratory failure (Polet et al, 1959).

In man, most instances of fatal intoxication have appeared to be primarily asphyxial; however, in others failure of both respiration and circulation have occurred together. It is likely that a sub-lethal degree of respiratory depression may be made lethal by associated reductions in blood pressure and cardiac output that can complicate gas exchange and nutrient delivery (DeCandole et al, 1953). In fatal poisoning by quaternary ammonium anti-ChE compounds (neostigmine, pyridostigmine) the onset of peripheral circulatory impairment appears earlier in the sequence of pathology (Merrill, 1948). Since this class of compounds passes the blood-brain barrier only very poorly, the central respiratory depression is delayed or absent.

The relative importance of central depression of respiration, peripheral neuromuscular block, and bronchoconstriction varies in different species and with different anti-ChE compounds and routes of administration (Barnes and Duff, 1953; DeCandole et al, 1953; Douglas and Mathews, 1952; Wright, 1954; Brimblecombe, 1977). With large doses there is usually a de-

crease in tidal volume and respiratory rate and an increase

in airway resistance. In man it is likely that failure of

the respiratory drive is the most important factor, with

peripheral neuromuscular block next in importance. Broncho-

constriction appears to be somewhat less severe in man than

in most animal species, although airway obstruction due to

copious secretions may also occur and may seriously impair

ventilatory exchange leading to asphyxia (GROB, 1963).

This obstruction may also countervene resuscitative efforts.

Effects on subjects with airways hyper-reactivity (asth-

matics) have not been reported.

> b) <u>Physiological Effects of Sublethal Doses of</u>
>
> <u>Anti-ChE Agents</u>

<u>General.</u> The pathophysiological effects of non-

lethal doses of anti-ChE agents are much less clear cut.

Generally the activities can be predicted on the basis of the

morphological distribution of cholinergic neural pathways

and the corresponding activities of the effector organs which

are so ennervated (Taylor, 1980). However, since cholinergic

synapses exist in preganglionic sympathetic, pre- and post-

ganglionic parasympathetic, neuromuscular, and CNS pathways,

the complexity of the response cannot be overstated. Potentially,

and depending on degree of inhibition, route of entry, and

specific agent activities, the anti-ChE agents can produce all

of the following effects (Taylor):

> (1) Stimulation, followed by depression or paralysis

of all muscarinic receptor responses at autonomic effector organs (e.g., vascular, airway, and gut smooth muscle; the heart; liver, gall bladder, spleen; the adrenal medulla; numerous secretory glands, etc.).

(2) Stimulation, followed by depression or paralysis of all autonomic ganglia and skeletal muscles (nicotinic actions).

(3) Stimulation, with subsequent depression of cholinergic receptor sites (primarily muscarinic) in the CNS (including the respiratory and cardiac control centers).

However, with relatively low doses, particularly those predicted for "respirator wearers" or those administered therapeutically, several modifying factors are significant. The response of effector organs to ChE inhibition depends on whether they receive cholinergic nerve impulses continuously or phasically. Compounds such as parathion must be biotransformed to paraoxon to elicit its inhibitory actions. Compounds that contain a quaternary ammonium group (neostigmine, pyridostigmine), do not readily pass the blood-brain barrier when present in low concentrations and thus their direct CNS activity is minimized (Schaumann, 1958). On the other hand, such compounds act relatively selectively at the neuromuscular junctions of skeletal muscle, exerting their action both as ChE inhibitors and as direct agonists (Riker and Wescoe, 1946). In contrast the more lipid soluble agents, such as tertiary amines (physostigmine) and most organophosphorous compounds,

have ubiquitous effects at both peripheral and central cholinergic receptor sites (Hobbiger, 1976; Palmer, 1980). In analyzing the peripheral effects of ChE, one must consider another complicating phenomenon: muscarinic agonists such as acetylcholine inhibit norepinephrine release (an adrenergic neurotransmitter) to the same organ (Loffelholz and Muscholl, 1969, 1970; Lindmar et al, 1968; Frossard and Muscholl, 1972; Vanhoutte, 1976; Muscholl et al, 1979), thus potentiating the imbalance between sympathetic-parasympathetic tone in those effector organs which are so ennervated. (e.g., the heart; Levy and Zieske, 1969).

Effects on Respiration. A number of investigators have reported that sublethal doses of physostigmine and neostigmine depress respiration in a variety of animal species, and concluded that their effects resulted from an inhibitory action on the central nervous system (medullary respiratory center), or on respiratory muscles, or both (Schweitzer and Wright, 1937, 1938; Feldberg and Sherwood, 1954; Schaumann, 1959).

In several studies, it was also found that an enhanced respiratory activity may be elicited by lower doses of physostigmine, and more frequently by neostigmine as well (Schweitzer and Wright, 1938; Erdmann et al 1955). Analyzing this apparent disparity in the literature, Weinstock et al (1981) pointed out that stimulation of respiratory activity was obtained in experiments where relatively small doses were administered to

unanaesthetized decerebrate animal preparations where the
inhibition level was not severe enough to depress the
respiratory muscles (Schweitzer and Wright, 1938; Erdmann
et al, 1955).

It has been shown that anaesthetic agents, particularly
pentobarbitone, can antagonize the stimulant effect of
acetylcholine on brain stem neurons (Bradley and Dray, 1973)
and even convert it to an inhibitory one. It therefore seems
probable that anaesthetic agents reduce the likelihood of
demonstrating a stimulant effect of low doses of anti-ChE
agents on respiration, and the value of an awake, normally
functioning animal preparation for study of autonomic
phenomena is emphasized.

The increase of respiratory rate in response to ChE
inhibition suggests a stimulant effect on the central respir-
atory center. Yet Schaumann (1959) and others have shown that
neostigmine, which can elicit this effect, does not readily pass
the blood-brain barrier, especially at the lower doses in
question. The respiratory center is, under normal circum-
stances, reflexly innervated by afferents from receptors in
the circulatory (aorta and carotid artery) and respiratory
systems (muscle spindles, Hering-Breuer reflexes), as well as
directly by neural pathways from higher nervous structures
(e.g., hypothalamus and voluntary pathways).

Inasmuch as the chemoreceptor area of the carotid body
contains acetylcholinesterase (Koelle, 1951) and nicotinic

receptors (Douglas, 1952; Hellstrom, 1977; Fitzgerald
et al, 1979) it seems reasonable to conclude that ChE
inhibition can stimulate or depress respiration reflexly
through its effects on the carotid body and other cholin-
ergic receptor sites. This view is supported by Weinstock
et al (1981) who indicates that physostigmine stimulates
the respiration of rabbits when administered in doses too
small to affect peripheral cholinergic function. In his
investigations he showed that the enhanced respiratory
activity was accompanied by a significant elevation of
arterial pH. Weinstock further showed that the dose of
neostigmine which was required to suppress plasma cholin-
esterase to the same degree as his previous experiments
with physostigmine, was not effective in changing res-
piratory parameters. A markedly higher amount of plasma
inhibition (and thus dosage), was required to stimulate
respiration, and this was accompanied by muscle fascicul-
ations and a fall in arterial pH (due to large increases
in blood lactate). Hexamethonium (a ganglionic blocking
agent) prevented the effect of neostigmine on respiration.
It was concluded, therefore, that physostigmine (a
tertiaryamine) stimulates respiration directly by its
effect at the medullary respiratory center, whereas neos-
tigmine (a quaternary ammonium compound which does not
readily pass into the brain) stimulates respiration re-
flexly by its excitatory effect on the carotid body afferents

(and possibly on other receptor areas). It is therefore possible that substances which pass the blood-brain barrier and affect the respiratory or other centers directly, as physostigmine and the organophosphorous compounds, may have an additional potentiating excitatory or inhibitory effect via peripheral chemoreceptors (Weinstock, 1981).

Another salient feature of anti-ChE poisoning in experimental animals is a diminished "inflatability (increased impedance to inspiration; including elastic and resistive forces) of the lungs." The effect has been observed in various species with such compounds as DFP (Model et al, 1946; Heymans and Jacob, 1947), HETP (Dayrit et al, 1946), TPP (Verbeke, 1949), and tabun (Holmstedt, 1951), and is often of such severity as to be suggested as the cause of death (Koelle and Gilman, 1949). This lessened distensibility is generally attributed to bronchoconstriction (DeCandole, 1953). DeCandole studied the bronchoconstriction effects of numerous anti-ChE compounds on a variety of species. The pattern to emerge was rather varied with detailed differences depending on the species and on the particular drug, as well as on the dose level used. Bronchoconstriction was seen in all species studied, but it was not considered to be the main cause of the failure of ventilation. With sarin, paraoxon, dyflos, and TEPP, the degree of bronchoconstriction at the time of acute respiratory failure was, for example, slight in rabbits, severe in cats, and often insignificant

in monkeys. It is generally accepted that the parasympathetic innervation of the lung via the vagus nerve is the principal motor control for both airway smooth muscle and glands (Widdicombe, 1963); and Vornanen and Tirri (1981) have shown that the trachea and bronchi are 10-100 times more responsive to cholinergic stimulation than the bronchioles or lung parenchyma and that this difference in sensitivity is probably due to a greater activity of cholinesterase in the large airway tissue. Measurements of lung compliance or airway resistance in guinea pigs in vivo suggest that the principal site of action of many drugs is at the level of the fine peripheral airways (Drazen and Austen, 1974). In dogs, the greatest part of total lung resistance (55%) is produced in 3-8mm diameter bronchi, due to strong vagal tone (Macklem et al, 1969). Thus, it is not surprising that the anti-ChE agents should exert their most striking constrictor effects in the large airways. In terms of explaining species differences of bronchoconstrictor effects, Rudolph et al (1958) have shown that there is a good correlation between abundance of peribronchial smooth muscle and severity of agent-induced resistance. Clements et al (1956) showed marked increases of respiratory resistance and decreases of respiratory compliance in dogs dosed with DFP or sarin. These changes were attributed to bronchoconstriction and further, these responses were accompanied by significant decreases in aerodynamic deadspace

that are the result of rather uniform constriction of the tracheo-bronchial tree. Others have shown that in cats, cholinergic agonists can also constrict the peripheral airways (respiratory bronchioles and alveolar ducts) with concomitant increase in small airways resistance and decreased compliance of the lung (Colebaten et al, 1966). Thus the entire lung may be effected by cholinergic agonists with differential effects varying with species, dose, and route of exposure.

The lacrimal, sweat, bronchial, salivary, gastric, and pancreatic gland cells all have cholinergic innervation, and are influenced by the anti-ChE agents. The respiratory significance of this activity lies in the fact that profuse secretions from the mouth, nose, eyes and bronchi can and do accumulate in the airways and can readily aggravate the respiratory distress produced by other mechanisms (Cullumbine and Dirnhuber, 1955), especially if some airways become completely occluded, altering the distribution of ventilation in the lung.

Effects on Circulation. All anti-ChE agents can cause, in most species, a slowing of the heart and a marked fall in the systemic blood pressure, but the detailed picture varies with the species studied, the depth and nature of any anesthesia present, the amount of agent administered, the presence and degree of hypoxia, and the underlying sympathetic tone (Verbeke, 1949; Holmstedt, 1951; DeCandole et al, 1953; Krop and Kunel, 1954;

Daly and Wright, 1956; Delga, 1957; Erdmann and Lendle, 1958; Levy, 1971). Very few studies have been performed with an awake, intact animal preparation, so much of the existing data is difficult to interpret. The fall in blood pressure may be preceded by a rise, especially with smaller doses, but many exceptions have been reported. In the rat, Sarin, DFP, physostigmine, TEPP, and Paraoxon have been shown to produce an increased heart rate and a sustained rise in blood pressure (Dirnhuber and Cullumbine, 1955; Varagic, 1955). Physostigmine in the spinal cat, and parathion, physostigmine and neostigmine in decerebrate cats, all produce a blood pressure increase (von Eickstedt et al, 1955). Sublethal doses of TEPP have been said to have a pressor effect in the dog (Paulet, 1954), while Heymans et al (1956) reported that tabun on sarin intravenously administered to chloralose-anesthetized dogs may induce either arterial hypotension or hypertension.

These diverse responses to anti-ChE agents suggest that these compounds are capable of producing a variety of effects depending on degree (% inactivation) and locus of enzyme binding. Thus central, reflex, and peripheral loci may be involved in determining the actual integrated cholinergic response by the cardiovascular system as a whole.

a) The Heart. It is generally recognized that the two divisions of the autonomic nervous system exert antagonistic effects on various aspects of the performance of the heart.

However, these opposing influences are not algebraicly additive; complicated interactions exist. Two major types of peripheral (at the heart) interactions have been described. The first type has been called "accentuated antagonism" between the sympathetic and parasympathetic control mechanisms (Levy, 1971). In the second type the peripheral components of one division are activated as a consequence of activity in the other; Levy calls this "reciprocal excitation". One of the earliest examples of the first type of interaction between sympathetic and vagal actions on the heart was described in 1934 by Rosenbleuth and Simeone. They observed that in anesthetized cats the absolute reduction in heart rate produced by a given vagal stimulus was considerably greater when the heart rate was elevated by sympathetic stimulation. These and many other similar results have been confirmed by more recent investigations and the extent of the interaction has been expressed quantitatively (Warner et al, 1969; Levy and Zieske, 1969). It is important to keep this interactive mechanism in mind as we review the effects of anti-cholinesterase agents on the heart.

As previously stated, the most common effect of anti-ChE agents on the heart is a slowing of the rate (bradycardia). Heymans et al (1956) have shown that this is due to a peripheral action. Thus, in the dog, the bradycardia persists after section of the cervical vagus nerves, and it is seen following

injection of tabun or sarin in the surviving decapitated trunks. Furthermore, injection into the circulation of the perfused isolated head, connected to its trunk only by the vagus nerves, does not induce bradycardia (Heymans, 1950). Therefore, these anti-ChE agents, unlike ACh, apparently do not stimulate directly the vagal cardioinhibitory center. Also, unlike ACh, they do not cause a reflex bradycardia by stimulation of the chemoreceptors of the carotid body, since injection into the circulation of the carotid body does not induce bradycardia; likewise it is not influenced by denervation of the carotid sinus (Heymans, 1951).

Ganglionic blocking agents, such as tetraethylammonium, azamethonium, and hexamethonium, given in sufficient doses to block the intracardiac vagal synapses, diminish the bradycardia response but a component still persists. This residual bradycardia can be abolished by atropine (Heymans et al, 1956; Holmstedt, 1951). Therefore the bradycardia is due, in part, to a nicotinic stimulant action on the parasympathetic ganglia, and in part, to a peripheral muscarinic effect on cardiac tissues directly.

Vagal stimulation and muscarinic cholinergic stimulation have been shown to alter ventricular function as well as heart rate (DeGeest et al, 1965; Dagget et al, 1967; Randall et al 1968; Wildenthal et al, 1969), exerting negative inotropic (Hollenberg et al, 1965), as well as chronotropic (Bailey et al, 1972; Tse et al, 1976) effects in the mammalian ventricle.

Furthermore, vagal stimulation due to muscarinic cholinergic agonists applied in the presence of simultaneous adrenergic stimulation results in a magnification of these negative inotropic and chronotropic effects (Levy et al, 1966, 1971; Dempsey and Cooper, 1969; Levy and Zieske 1969; Bailey et al, 1979). This latter phenomenon may have important implications if an exercising organism is exposed to anti-ChE agents since the exercise-induced high levels of adrenergic tone may potentiate the vagal effects.

b) The Systemic Circulation. Hypotension is commonly seen after the administration of adequate doses of anti-ChE agents in most species. This could be due to a decreased cardiac output resulting from vagally depressed cardiac function, to a decreased peripheral resistance, or both.

Verbeke (1949) using HETP and TEPP in the dog attributed the resultant fall in blood pressure to peripheral vasodilation. Holmstedt (1951), on the other hand, reported an increased total peripheral resistance following infusion of tabun into the rabbit. Paulet (1954) concluded that the pressor action of small doses of TEPP that he observed in the dog was due to actions on the sympathetic ganglia, adrenals, and medullary centers. Daly and Wright (1956) also believed that the vasoconstriction action of anti-ChE agents might be caused by liberation of sympathomimetic substances from the adrenal glands. Such an action was first demonstrated by Stewart and Rogoff (1921) using physostigmine in the cat.

When the vasoconstrictor mechanisms in the limb are excluded by dissection of the nerves to the limb and by removal of the adrenal glands, a vasodilator effect of the anti-ChE agents is unmasked. Thus Daly and Wright (1956) found that injection of sarin caused vasodilation in cross-perfusion experiments when the limb was perfused from an adrenalectomized donor. This effect must have been due to agents carried by the blood, and the likely explanation is the accumulation of endogenous ACh which has long been known to produce vaso-dilation (Dale, 1914).

There have been somewhat more recent reports that neostigmine elevates blood pressure and heart rate in anesthetized ganglion blocked dogs (Long and Eckstein, 1961; Hilton, 1968). Levy and Ahlquist (1962) found that neostigmine lowered heart rate and blood pressure in anaesthetized dogs without ganglion blockade.

The effect of anti-ChE agents on the systemic peripheral circulation is, thus, a complex one. Vasoconstriction may be produced by several indirect mechanisms; vasodilatation may be caused by local actions. Uniformity of response under different experimental or operational conditions, therefore, cannot be expected (Cullumbine, 1963), since these responses are dependent on such variables as species, dose, route of entry, state of consciousness, and underlying sympathetic tone.

Effects on Skeletal Muscle. Most of the effects of potent anti-ChE compounds on skeletal muscle can be explained on the basis of inhibition of acetylcholinesterase at the neuro-muscular

junction. However, evidence suggests the existence of a direct action of the quaternary ammonium agents on skeletal muscle fibers as well (neostigmine, pyridostigmine). For example, Kiker and Wescoe (1946) showed that intra-arterial injection of neostigmine into chronically denervated muscle, or into normally innervated, but AChE inactivated muscle, evokes an immediate contraction, whereas physostigmine (a tertiary amine) does not.

Normally a single nerve impulse in a terminal motor axon branch liberates enough ACh to produce a localized depolarization to end-plate potential, of sufficient magnitude to initiate a propagated muscle action potential. The ACh released is rapidly hydrolyzed by AChE, such that the lifetime of free ACh within the synapse is shorter than the decay of the end-plate potential or the refractory period of the muscle (Colquin, 1979). Therefore, each motor impulse gives rise to a single wave of depolarization. After inhibition of AChE the residence time of ACh in the synapse increases and therefore the probability of ACh rebinding to the receptors is also increased and a prolongation of the decay of the end-plate potential is observed. Quanta released by individual nerve impulses are thus no longer isolated and well-defined. This destroys the synchrony between end-plate depolarization and the development of the action potential. Consequently, asynchronous excitation and fibrillation of muscle fibers are observed (fasciculations). When ACh persists in the synapse, it may also act on the axon terminal, resulting in

anti-dromic firing of the neuron; this contributes to the fasciculations which involve the entire motor unit. With sufficient inhibition of AChE, depolarization of the end-plate predominates and blockade due to depolarization ensues. Thus, a small dose of physostigmine or neostigmine may increase the skeletal muscle contraction produced by a single maximal nerve stimulus, but larger doses or repetitive nerve stimulation at a rapid rate may result in depression of "block" (Taylor, 1980).

Thus it can be visualized that a given muscle or group of muscles can react very differently, even oppositely, to a given anti-ChE compound depending on the solubility of the agent, the dose, the route of entry, and the existing state of neural stimulation. The range of responses includes excitation and enhanced strength and contractility to depressed function, muscular weakness, and paralysis. Predictive ability of the potential for respiratory muscular fatigue or paralysis, relative to dosage or degree of cholinesterase inhibition, would be a matter of considerable interest to those who are subject to exposure to these compounds. Yet most reports on toxic manifestations of the anti-ChE's deal almost exclusively with the central respiratory effects of lethal doses, and very little is known concerning the progression of events that precedes muscular respiratory failure. There is a substantial need to characterize this onset with quantifiable cholinesterase inhibition-activity data and to integrate this knowledge with the known cardiovascular and respiratory functional decrement associated with these compounds.

2. The Physiological Effects of Resistance Loading

There are several reasons why researchers have studied respiratory mechanical loading; first, to elucidate basic physiology; second, to improve understanding and treatment of the disturbances in patients with respiratory diseases; and third, to assist in the design of breathing equipment for medical and respiratory protection purposes (Milic-Emili, 1970).

Respiratory loads can be external (i.e., applied mechanically to the airway openings or to the chest or abdominal wall) or internal (i.e., within the respiratory system). Loads can be inspiratory, expiratory, or continuous if applied to both phases of the breathing cycle. They can be elastic, flow resistive, or inertial depending on whether they are related to volume, flow, or acceleration and they may be linear or non-linear as they act across the physiological range of respiratory parameters. Application of constant pressures at the airway opening or at the body surface have been referred to as pressure biasing (Mead, 1979), and these too may be either inspiratory, expiratory, or continuous. A recent review by Cherniak and Altose (1981) deals with respiratory responses to the entire spectrum of mechanical loads. This present review will be primarily confined to resistive loading of inspiration and will depict not only the ventilatory responses, but also the integrated circulatory responses, blood gas changes, and oxygen cost of loaded breathing as well.

Breathing Responses to Inspiratory Resistance Loading. In
anesthetized animal preparations, the addition of inspiratory
loads does not substantially affect the overall intensity of
inspiratory muscle activity, except for prolonging it. There
is ample evidence that in anesthetized animals the addition of
external loads (including airway occlusion at end-expiration)
does not affect the time course of the diaphragmatic and phrenic
discharge except for prolonging it (Cohen, 1979; Younes et al,
1974, 1981). On the other hand, external inspiratory loading
often elicits an immediate reflex increase in the activity of
the external intercostals and accessory muscles (Corda et al,
1965), but these accessory reflexes are thought to be of little
importance in terms of load compensation in anesthetized prep-
arations except that they may present an additional oxygen
demand of breathing. Accordingly, in anesthetized preparations
the main immediate neural mechanism for stabilizing tidal volume
in the face of mechanical inspiratory loads in general is
represented by prolongation of inspiratory time (T_I). This
reflects the prolongation of electrical discharge (onset to peak)
of the diaphragm, external intercostals and accessory muscles,
and in the nerves supplying them which is elicited via the
Breuer-Hering inflation reflex (Cohen, 1979; Siafakas et al, 1981;
Younes, 1974). With added flow resistance, however, a further
prolongation of inspiratory time (T_I) is caused by the time lag
between neural drive and volume change (Miserocchi et al, 1976).
Indeed, the translation of neural input into mechanical output

depends on a chain of events: nerves stimulate muscles, muscles move the chest wall and lung, and lung moves gas (Mead, 1979; Milic-Emili, 1981). This process involves a time constant which can be thought of as being the time constant of the respiratory system (τrs). It is important to note that the time lag between neuromuscular drive and volume change depends not only on τrs, but also on the magnitude and rate of decay of post-inspiratory activity of the diaphragm and other inspiratory muscles. This is the activity exhibited after peak intensity of discharge is reached and which normally extends well into the spirometer expiratory phase (Green and Howell, 1959; Petit et al, 1960).

On the basis of the above considerations, it follows that the addition of flow-resistive loads should result in a more marked prolongation of spirometric inspiration than is the case for other mechanical loads (i.e., elastic). And, indeed, this has been shown in anesthetized cats by Miserocchi and Milic-Emili (1976), and that in many cases the prolongation of T_I is sufficient to maintain tidal volume (V_T) nearly constant in the fact of substantial added resistive loads.

Although the Breuer-Hering inflation reflex can provide substantial stability of V_T, this is not the case in terms of minute ventilation. Indeed, the prolongation of T_I, with resistive loads, mediated by this reflex mechanism, necessarily implies a reduction of V_T/T_I, and ventilation ($\dot{V}_E$) will only be stabilized if T_I/T_{tot} increases with loading. This can be shown

readily by Milic-Emili's (1976) analysis of the breathing

cycle which describes ventilation as being the resultant of

a driving and a timing mechanism:

$$\dot{V}_E = (V_T/T_I) \times (T_I/T_{tot})$$

where V_T/T_I is the mean inspiratory flow, and T_I/T_{tot} is the ratio

of inspiratory to total breathing cycle duration and has been

termed the inspiratory duty cycle (Wyszogrodski et al, 1978).

The advantage of this equation for analyzing the breathing cycle

is that ventilation is partitioned into two components; one

reflecting timing (T_I/T_{tot}) and the other being related to

neuromuscular inspiratory drive (V_T/T_I) and the operating

characteristics of the respiratory system (active impedance and

operating length of inspiratory muscles). It should be noted,

however, that V_T/T_I may also exhibit marked dependence on in-

spiratory time (T_I), particularly when the volume time-course

of inspiration deviates substantially from a straight line

(Milic-Emili and Zin, 1981; Sifakas et al, 1981). This approach

is particularly useful in analysis of the effects of resistance

loading on ventilation. For example, the prolongation of T_I,

such as is often elicited by an added flow resistance, will

defend V_T but not necessarily V_T/T_I, and if the load causes a

proportional increase of T_I and T_E (expiratory time), T_I/T_{tot} will

be unchanged.

Accordingly, the effects of inspiratory loads on timing

have to be considered. While the immediate effects of loads on

V_T have been extensively studied in a variety of experimental

animals and in humans, less attention has been paid to
timing. In most intact animals, the frequency of breathing
(f) decreases with flow-resistances added during inspiration
or expiration or throughout the breathing cycle (Bruce et al,
1974; Campbell et al, 1964; Davenport et al, 1981; Koehler
and Bishop, 1979; Lynne-Davies et al, 1971, 1974, 1975;
McClelland et al, 1972; Nunn and Ezi-Ashi, 1961; Zechman
et al, 1976). As a result, ventilation is less well
compensated than V_T.

According to Grunstein et al (1974), in terms of the ability
of the respiratory system to stabilize ventilation during
inspiratory elastic loading, little difference exists between
results obtained in anesthetized cats before and after
unilateral or bilateral vagotomy. In fact, inspiratory elastic
loading appears to have very little effect on T_I/T_{tot} (Miserocchi
and Milic-Emili, 1976). On the other hand T_I/T_{tot} tends to
increase with flow-resistive loading as a result of dispro-
portionate prolongation of T_I relative to T_E due to an increased
respiratory system time constant (rs), and this increase is
more marked when flow resistance loads are added during in-
spiration alone (Miserocchi and Milic-Emili, 1976; Zechman
et al, 1976); and this response too, remains after bilateral
vagotomy.

Thus, the immediate response to elastic and flow-resistive
loading is a reduction in V_T/T_I (mean inspiratory flow). In the
case of added inspiratory flow-resistances this response is

compensated to a significant degree by a concomitant increase in T_I/T_{tot}, which defends ventilation. During sustained loading, there is a progressive increase in ventilation after the initial drop (Campbell et al, 1961, 1964) which never returns to the preloaded values. In most preparations virtually all of the progressive increase result from changes in chemical drive, and its magnitude depends on the sensitivity of peripheral and central chemoreceptors. Although there is abundant evidence suggesting that during respiratory loading changes in arterial PO_2 and PCO_2 occur quite rapidly, accounting for the progressive increase in respiratory activity after the first loaded breath (Bruce et al, 1974; Orthner and Yamamoto, 1974), other contributory mechanisms have been proposed, such as increased afferent discharge from lung and chest wall receptors (Campbell et al, 1961; Kelsen et al, 1976).

If respiratory loading causes an increase in end-expiratory lung volume, the operating length of the inspiratory muscles will decrease, resulting in decreased effectiveness of their contraction in terms of pressure generation (Marshall, 1962; Pengelly et al, 1971). This phenomenon is usually observed with expiratory loads and it generally elicits recruitment of expiratory muscle activity via a vagally mediated reflex (Koehler and Bishop, 1979), and implies an additional energy expenditure (work of breathing) during what is normally a passive maneuver at rest.

During sustained respiratory loading the differences in

response between the awake and the anesthetized states are very marked. First, in awake subjects ventilation tends to return more quickly toward control values than during anesthesia (Margaria et al, 1973). Second, in awake subjects under steady state conditions there is little change of alveolar gas composition even in the face of substantial flow-resistive loads (Agostini et al, 1978; Zechman et al, 1957). This implies that non-chemical neural load compensatory mechanisms are more effective during wakefulness.

In awake states the responses to loading can be modified by anticipation and previous experience, and may depend on individual traits (Aitken et al, 1970; Freedman and Weinstein, 1965). In conscious subjects sustained external flow-resistive loads cause markedly decreased frequency of breathing (Freedman 1969, 1970; Zechman et al, 1957). By contrast, in anesthetized subjects the changes in respiratory frequency are small or absent independent of the type of external loading (Freedman 1969, 1970; Margaria, 1973). The response of awake normal individuals appears to be useful in terms of minimization of mechanical work of breathing (Mead, 1960; Otis et al, 1950). In this connection, it should be noted that in awake patients with respiratory mechanical disorders, a decreased frequency of breathing is seldom seen even in the presence of markedly severe internal flow resistance (Milic-Emili, 1982). This implies that internal respiratory loading is not necessarily analagous to external loading.

The respiratory system has a remarkable capacity to maintain ventilation within fairly narrow limits despite considerable changes in mechanical loading. This ventilatory stability is provided by three main mechanisms: (a) the chemoreceptor-respiratory center control system; (b) nervous reflexes originating from pulmonary receptors and chest wall proprioceptors (most of which have cholinergic components); and (c) mechanisms intrinsic to the ventilatory pump. The latter include the intrinsic properties of the respiratory muscles (force-length, and force-velocity relationships), their mechanical arrangement and geometry, and the mechanical properties of the various structures comprising the ventilatory apparatus. In conscious subjects behavioral factors can significantly modify the type and degree of breathing responses to loading (e.g., experience, training, etc.), and perceptions of comfort or dyspnea may confound the "typical" ventilatory response (Raven, 1979).

Effects of Resistance Loading on Ventilatory Responses to Exercise. Changes in airway resistance also affect the level of ventilation during exercise. Breathing a low density helium-oxygen gas mixture which reduces the turbulent resistance to airflow increases ventilation during exercise (Nattie and Tenney, 1970), whereas inspiratory flow-resistive loading decreases ventilation during exercise (Flook and Kelman, 1973). Changes in airflow resistance primarily affect breathing frequency rather than tidal volume. Moderate inspiratory flow-resistive

loads prolonged inspiratory duration, but tidal volume was actually increased (Flook and Kelman, 1973). Only during heavy exercise did tidal volume fall during flow-resistive loading. Similarly, breathing a helium-oxygen gas mixture shortened both inspiratory and expiratory time but did not affect tidal volume (Nattie and Tenney, 1970).

Normally, during exercise, tidal volume and breathing frequency increase together until tidal volume reaches a certain maximal value. Thereafter, further changes in ventilation result from increasing respiratory frequency alone (Cotes et al, 1970). Elastic loading, for example, reduces the vital capacity and decreases the maximal tidal volume reached during exercise. Consequently, during elastic loading, any given ventilation during exercise is achieved by a smaller tidal volume and a more rapid breathing frequency than during free unloaded breathing (Cotes et al, 1970). In contrast, flow-resistive loading has no effect on the maximal tidal volume but tends to limit the increase in respiratory frequency. Neither kind of load affected the relationship between ventilation and tidal volume during moderate exercise despite their differing effects at rest. These findings contrast with the idea that breathing patterns are set to minimize work expenditure.

Demedts and Anthonisen (1973) have shown that exercise performance is progressively reduced by flow-resistive loads of increasing severity. Flow-resistive loading reduced the 15-sec

maximum voluntary ventilation at rest and correspondingly
reduced the maximum ventilation during exercise. However,
during flow-resistive loading, the maximum ventilation during
exercise remained fixed at approximately 70% of the maximum
voluntary ventilation at the same level of airway resistance.
Cerretelli et al (1969) showed that exercise could be sustained
during flow-resistive loading only as long as swings in mouth
pressure during breathing remained below 100 cmH$_2$0. However,
Demedts and Anthonisen (1973) noted that maximum mouth pressure
swings were much lower in some subjects and were inversely
related to the PCO$_2$ during maximum exercise. They further
demonstrated that those individuals with the lowest ventilatory
responses to CO$_2$ had the smallest swings in mouth pressure.
These subjects seemingly chose to allow PCO$_2$ to rise by
minimizing their ventilatory efforts and were able to tolerate
higher levels of exercise during flow-resistive loading. On the
other hand, individuals with high ventilatory responses to CO$_2$
had large swings in mouth pressure. By expending large
respiratory efforts, they were able to maintain PCO$_2$ at near
normal levels but could tolerate lesser degrees of exercise.

The study of Demedts and Anthonisen (1973) confirmed the
previous observations of Milic-Emili and Tyler (1963) that during
rest the relation.hip of PCO$_2$ and the work rate of the inspiratory
muscle is not affected by low-resistive loading. This constant
relationship, however, does not hold at high levels of exercise;
this can be explained by the observations of Cerretelli et al (1969),

who found that the total respiratory work rate during exercise and flow-resistive loading is equal to the sum of two factors - one proportional to the exercise level and the other proportional to the PCO_2. It thus seems that during exercise the responses to flow-resistive loading are less dependent on the relationship between PCO_2 and work of breathing.

What factors determine the limit of tolerance to resistance breathing during exercise is not clear. It has been suggested that the limit is determined by the force of contraction of the respiratory muscles as reflected by the peak mouth pressure rather than by the overall work of breathing (Cerretelli et al, 1969; Demedts and Anthonisen, 1973). However, several investigators have reported decreased oxygen consumption ($\dot{V}O_2$) after resistance loading and have suggested the tendency to develop anaerobic metabolism due to compromised pulmonary gas exchange (Thompson and Sharby, 1966; Hanson et al, 1965; Cerretelli et al, 1970). Some controversy on this point exists, however, since Gee et al (1968) suggested that $\dot{V}O_2$ did not change after resistance loading.

Circulatory Responses to Inspiratory Resistance Loading. Reports of cardiovascular changes associated with the application of inspiratory resistances are scarce and conflicting. Elevated inspiratory resistance was reported to elevate heart rate (Spioch et al, 1962) to lower heart rate (Van Huss et al, 1967), to lower heart rate and cardiac output (Shephard, 1962), and

to keep heart rate unchanged (Chatterje 1969, Flook & Kellman, 1973). It is not clear whether the pattern of heart rate change is dependent upon the pattern of ventilation or not. It it likely however that if there are consistent circulatory changes, they might be caused by reflexes rather than by direct mechanical effects.

Lung inflation and deflation and the changing level of pulmonary stretch receptor activity have been shown to cause swings in blood pressure, heart rate, and cardiac output that are synchronous with the breathing cycle, even during normal quiet breathing (Cherniak et al, 1969; Glock et al, 1969; Hainsworth, 1974). These circulatory changes may be exaggerated or otherwise modified during ventilatory loading due to alterations in the end-expiratory position of the chest wall. Pulmonary stretch receptor stimulation has been shown to produce systemic vasodilatation and tachycardia, whereas stimulation of irritant receptors results in vaso-constriction. Increased activity of the J-receptors causes bradycardia (Paintal 1969, 1973; Widdicombe, 1974). The overall circulatory effects of ventilatory loading depend on the nature and degree of the load, ce one type of receptor may be stimulated to a greate, or lesser degree than others (Hainsworth, 1974).

The subatmospheric intrathoracic pressure swings become exaggerated during inspiratory loading. Right ventricular output is augmented, but the impedance to left ventricular

ejection (afterload) is increased (Charlier 1974; Wise et al,
1981). The resulting elevation of intrathoracic blood volume
secondary to increased afterload, may cause some degree of
interstitial or peribronchial congestion which could act
to stimulate the J-type and irritant receptors of the lung
(Cherniak and Altose, 1981); or it could increase peripheral
airway resistance by competition for space within the peri-
bronchial sheath (Hagg et al, 1971).

Both positive and negative pressure breathing produce
serious circulatory consequences, and in terms of their effect
on intrathoracic pressure swings are somewhat analagous to
expiratory and inspiratory resistance loading. Cruz et al
(1967) and Baer and Ehrlich (1978) showed significant decreases
in cardiac output in response to expiratory loading as a result
of the diminished gradient for venous return to the heart
that is induced by the increased expiratory time and elevated
intrathoracic pressure during loading.

Respiratory adjustments during ventilatory loading can
also be mediated by changes in the activity of carotid or
aortic baroreceptors. Baroreceptor stimulation inhibits in-
spiration (Grunstein et al, 1975; Bishop 1974). Conversely,
positive pressure breathing, by decreasing baroreceptor discharge
consequent to hypotension, excites inspiratory activity
(Bishop 1968, 1974). The fall in baroreceptor discharge plus
the increased stretch receptor activity produced by lung
inflation would also serve to stimulate expiratory activity

and cause the abdominal muscles to actively contract. These actions would augment venous return and restore the blood pressure by the concomitant increase in cardiac output (Bjurstedt, 1953). Since baroreceptor discharge also decreases the activity of peripheral chemoreceptors, positive pressure loading, through its inhibitory effect on the baroreceptors, could also augment the ventilatory responses to chemoreceptor stimulation (Heistad et al, 1975).

Oxygen Cost of Inspiratory Loaded Breathing. The muscles of respiration comprise three groups: The diaphragm, the intercostal and accessory muscles, and the muscles of the abdomen. All three groups have inspiratory and expiratory function and work together in intricate ways. When loaded, the inspiratory muscles used to expand the chest and lower the diaphragm during the breathing cycle can fail in much the same manner and for the same reasons as does the myocardium in heart failure (Roussos and Macklem, 1977; Macklem and Roussos, 1977; Roussos et al, 1979). When the inspiratory muscles' demands for energy exceed their supply, the energy stored within the muscles is depleted and the force of contraction diminishes. This condition is known as inspiratory muscle fatigue; and when it occurs alveolar ventilation decreases, the arterial carbon dioxide tension ($PaCO_2$) increases, and hypercapnic respiratory failure may ensue (Anderson et al, 1978).

The greater the work required for breathing, the greater is the energy demand. Thus, an increase in the work of breathing will

predispose to fatigue (Roussos et al, 1979). The factors
that determine the work of breathing include:

1) Minute ventilation. Any increase in ventilation
increases the work of breathing.

2) Rate and depth of breathing. The optimum rate and
depth of breathing for any given level of alveolar ventilation
is determined by the compliance and resistance of the lungs
and chest wall (McIlroy et al, 1954). Any deviation from the
optimum, with either an increased rate and a decreased tidal
volume or a decreased rate and an increased tidal volume, will
increase the work of breathing.

3) Compliance and resistance of the lungs and chest wall.
Stiff lungs with low compliance, and airway obstruction with
high resistance both increase the work of breathing (including
externally applied resistance to breathing).

The strength of the inspiratory muscles can also effect
energy demands. A weak muscle requires more energy in relation
to its maximum energy consumption to do a given amount of work.
The force developed by a skeletal muscle that is sufficient
to produce fatigue is a function of the maximum force the
muscle can develop. Thus, for an isometrically contracting
muscle the force of contraction must be 15% or more of the
maximum to result in fatigue. For an intermittently contracting
muscle like the diaphragm, the critical force is about 40% of
the maximum (Roussos and Macklem 1977; Bannister and Brown, 1968).
Any condition that decreases the maximum force will predispose

to fatigue and reduced muscle function. Such conditions include atrophy, immaturity, performance on an inefficient part of the muscles' length-tension characteristics, or neuromuscular weakness due to compromised motor end-plate transmission. The maximum force a muscle can develop is a function of fiber length. The fibers of the inspiratory muscles shorten as the lung volume increases. Thus, hyperinflation leads to a decrease in fiber length and a decrease in muscle strength, predisposing to fatigue (Roussos et al, 1979). Inspiratory resistance loading, as has been previously mentioned, can produce significant alterations in the rate and depth of breathing as well as imposing the extra work requirements of the resistance itself, producing the synergic potential for early respiratory failure.

Finally, muscle efficiency, the ratio of external work performed to energy consumed, is an important factor in energy demands. Inspiratory muscle efficiency is known to fall as the breathing resistance increases (McGregor and Becklake, 1961), probably because the diaphragm behaves more as a fixator and less as an agonist (Macklem et al, 1978) under these load conditions. A fixator contracts isometrically and by definition does not change length. Under these circumstances the diaphragm can perform no external work, and as it continues to consume oxygen its efficiency falls to zero.

If efficiency remained constant in different conditions of loaded breathing, external work rate of breathing ($\dot{W}_b$)

could be used as a predictor of the oxygen cost of breathing
(O_2CB). However, respiratory muscle efficiency has proven
to be a parameter with quite wide variation, ranging from
1 to 25% (Campbell et al, 1957; Cherniak, 1959; McGregor
and Becklake, 1961). The wide range of respiratory muscle
efficiencies may be related to the fact that "Work", as
conventionally measured (Campbell, 1970), does not represent
or adequately reflect all the oxygen consumed by these
muscles. To this end, McGregor and Becklake (1961) showed
that respiratory muscle force was a better estimator of
O_2CB than was work. Rochester and Bettini (1976) found a
strong linear relationship between their pleural pressure-
time index (product of the average area contained by the
pleural pressure trace during inspiration, and the respiratory
rate), and the oxygen consumption of the diaphragm in dogs
with open abdomens. Both force and pressure reflect tension
development by the respiratory muscles rather than the work
they perform. Rochester and Bettini (1976) also showed that
in the diaphragm during increases of inspiratory resistance
loading, the oxygen requirements are met by an increase in
blood flow and an increase in oxygen extraction. In this
regard the diaphragm resembles other skeletal muscles
(Mottram, 1958). However, at higher levels of tension
development, extraction of oxygen by the diaphragm tended
to plateau whereas diaphragmatic blood flow continued to rise.
This behavior is unlike limb skeletal muscle and suggests that

the diaphragm resembles the heart in that it depends on
perfusion to meet its oxygen demands. The biochemical
properties of the diaphragm are also more like the heart
than limb skeletal muscle in that its enzymes heavily
favor aerobic metabolism (George et al, 1961; Kar and
Pearson, 1963). Thus, both the vascular and biochemical
components of the diaphragm are apparently well suited for
endurance work as long as adequate perfusion is supplied.
On the other hand, Aubier et al (1981) demonstrated clearly
that respiratory muscle fatigue leading to complete failure,
accompanies the decreased cardiac output of experimental
pericardial tamponade. They suggested that failure of the
respiratory muscle contractile mechanisms was brought on
by an increasing ventilatory workload in the face of
diminishing availability of aerobic fuels for muscular work
caused by the diminished cardiac output. They dismissed the
role of compromised central respiratory drive in the observed
respiratory failure, since integrated electrical output of
the phrenic nerve and diaphragmatic EMG increased until the
point of death.

It is not difficult to extrapolate the inferences drawn
from Aubier's results to the scenario, where an external
mechanical load (respiratory protective mask) placed on the
respiratory musculature during conditions of cholinergically
diminished cardiac output, could result in a similar pro-
gression of events leading to respiratory failure.

C. Summary

In the foregoing review the nature and magnitude of compensatory mechanisms invoked by external inspiratory resistance loading have been presented. And with the potential for interaction with these mechanisms in mind, the respiratory and circulatory pathophysiology of a family of toxic chemicals, known broadly as the anticholinesterase agents, was explored.

Briefly, the breathing responses to resistance loading were shown to involve mechanical and neuromuscular mechanisms that involve vagal reflex mediation through the Hering-Breuer reflexes, muscle spindles, and other autonomically innervated pulmonary receptors, as well as through perceptual higher brain center activities. If these compensatory changes result in altered blood gas tensions, then control of breathing via the peripheral chemoreceptors may also be invoked by the resistance loading, indirectly. Circulatory changes, such as blood pressure, heart rate, and cardiac output were shown to be e ected synchronously by stimulation of pulmonary mechanoreceptors secondary to resistance loading, but controversy exists on which receptors play dominant roles.

All these ventilatory and circulatory response mechanisms, mediated through efferents and afferents in the parasympathetic trunks of the vagus nerve, are either exclusively cholinergic or have significant cholinergic components. Thus, it is readily

apparent that there exists a potential for interaction, between these cholinergic cardiopulmonary compensatory mechanisms and an environmental contaminant whose toxic effects are manifested in these same autonomic and neuro-muscular systems (i.e., anti-ChE agents). What is not clear, however, is the mechanistic nature of those interactions which result, and to what extent they will compromise the compensatory mechanisms invoked by inspiratory resistance loading, and to what extent cardiopulmonary function in general will be compromised. Neither is it clear if these integrated response mechanisms will become further disrupted during even a mild exercise challenge, when the "fine-tuning" of autonomic reflex control mechanisms becomes critical.

The following experimental design utilizing an awake, chronically-instrumented mongrel dog preparation, was con-structed to address these voids in our understanding. For the first time an intact, awake, exercising animal model with a comprehensive array of cardiopulmonary monitors, will be used to study the integrated cardiopulmonary responses to environmental stresses applied separately and in simultaneous combinations. The potential data generated by such a model is vast and is amenable to application to a broad range of physiological questions. However, the foregoing literature review has generated the following specific questions that will be addressed by this investigation:

Resistance Loading.

(1) What is the effect of inspiratory resistance loading on the heart and circulation? Are the changes involved reflex, mechanical, or a result of the metabolic demands of the muscles powering the ventilatory "pump"?

(2) What is the magnitude of the mechanical load on the respiratory muscles? Is there any evidence of incipient respiratory muscle fatigue from the mask's resistance?

(3) Is pulmonary gas exchange impaired by the respirator mask-induced changes in circulatory and ventilatory mechanics?

Cholinesterase Inhibition.

(1) It is known that large doses of anti-ChE agents depress the respiratory center in the brain directly; but what is the integrated response of the respiratory system to moderate peripheral (non-CNS) cholinesterase inhibition? Are the responses essentially mechanical, as induced by bronchoconstriction and other cholinergic airway resistance increases; or are they reflex in their mechanism of control?

(2) What is the magnitude of the cholinergic resistance load on the respiratory muscles? Is there any evidence that the respiratory muscles have been compromised (by neuro-muscular cholinergic insult) in their ability to ventilate the lungs under these conditions of increased muscular workload?

(3) What is the integrated response of the cardiovascular system to moderate peripheral cholinesterase inhibition? Is

cardiac function depressed and by what mechanisms? Is there a "pressor" response from an exclusively peripheral cholinesterase inhibitor?

(4) If respiratory workload goes up and cardiac function is depressed, how is this imbalance maintained?

Interactions

(1) Are the challenge conditions of inspiratory resistance loading and cholinesterase inhibition qualitatively additive or synergistic in their effects on cardiopulmonary functional mechanisms?

(2) Does the additional physiological burden of mild exercise alter the cardiopulmonary compensatory response mechanisms that were observed at rest? If so, does this interaction result from mechanical or autonomic reflex mediation?

(3) When all the challenge loads of inspiratory resistance, peripheral cholinesterase inhibition, and exercise are applied simultaneously, is there evidence of inadequate ability to compensate for these loads? Are there signs of respiratory muscle fatigue, impaired gas exchange, or inadequate cardiac output to the exercising muscle beds?

(4) Are the three environmental stresses simultaneously tolerable? For how long?

CHAPTER II.   METHODS

A.   Surgical Preparation

Seven mongrel dogs weighing between 17 and 22 kg were
surgically instrumented to record selected cardiopulmonary
functions.   Prior to instrumentation the dogs were
trained in the experimental environment to tolerate the
requisite experimental conditions (i.e., respiratory
mask, exercise on an inclined treadmill, and moderate
ChE inhibition).

The dogs were surgical instrumented with sterilized
pressure and flow sensors under aseptic conditions.   The
animals were anesthetized with pentobarbital sodium
(30 mg/kg, I.V., supplemented as necessary).   Ventilation
was maintained during the surgical procedure by a Harvard
respirator pump after endotracheal intubation (pump settings:
375 ml - 425 ml = tidal volume; frequency = 20/minute).

The surgical procedure was carried out in two stages.
First, the chest was opened in the fifth intercostal space.
The chest wall musculature was separated rather than cut
whenever possible.   After retraction of the ribs, a 16-
gauge polyvinyl catheter was introduced into the left atrium
via the pulmonary vein of the left middle lobe of the lung.

The pericardium was transected ventral to the phrenic
nerve and an appropriately sized Biotronex electromagnetic

flow transducer was fitted around the aorta adjacent to the pulmonary artery. To accomplish this installation, the aorta was dissected away from the pulmonary artery and a sheath of Marlex mesh was placed surrounding the para-aortic fat body and the aortic root between the flow probe and the vessel. This procedure was included to minimize the later erosion and rupture of the aortic wall which frequently accompanies chronic implantations of this type.

Finally, a pleural pressure valve of the type described by Tosev et al (1969) was secured in the seventh intercostal space at the midthoracic level. The pericardium was sutured shut except for a small opening allowing for the exit of the flow probe lead. A chest drain was inserted in the sixth intercostal space and the various leads from the implanted sensors were brought subcutaneously out through the dog's skin along the dorsal midline. The separate muscle and cutaneous layers were closed independently and the chest was evacuated by inflating the lungs, maintaining negative pressure on the chest tube, and closing the tube canal with a purse string suture. This concluded the first stage of the surgical procedure.

The second stage of the procedure entailed making an incision in the left axilla parallel and adjacent to the insertion of the pectoral muscle. The underlying muscle was separated without cutting to expose the left axillary artery
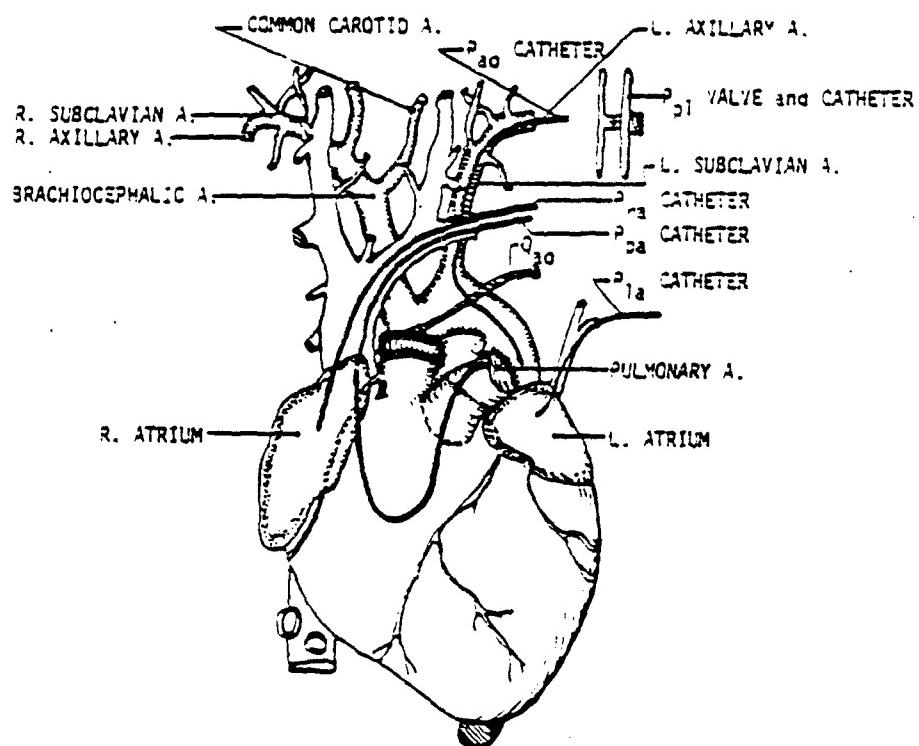
Figure II-A.   Circulatory Sensors

Placement of the circulatory pressure catheters and electromagnetic flow probe in and around the heart: aortic pressure (P_{ao}), left atrial pressure (P_{LA}), right atrial pressure (P_{RA}), pulmonary artery pressure (P_{PA}), and aortic flow ($\dot{Q}_{ao}$) are shown.

and vein. A 16-gauge polyvinyl catheter was threaded into the descending thoracic aorta via the left axillary artery. Two additional 16-gauge polyvinyl catheters were inserted via the axillary vein into the right atrium and into the pulmonary artery. Fluoroscopy was used to insure the accurate placement of the catheter tips at the desired locations. Optimally, the right atrial catheter should rest at the junction of the venae cavae with the right atrium, and the pulmonary artery catheter tip should extend several centimeters beyond the pulmonic valve but not so far as to impinge on the vessel wall at a bifurcation. Furthermore, looping of the pulmonary artery catheter in the right ventricle was avoided, since subsequent uncoiling generally results in the catheter tip moving and becoming wedged on the vessel wall deep within the lung, thereby clamping the pressure signal. The catheters were secured to the vessels at the axillary incision and threaded subcutaneously out through the back adjacent to the sensor leads that had been exited during the first stage of the surgical preparation. The incision was then closed by layers, concluding the surgical procedures. The various catheter and flow transducer locations are shown diagrammatically in figure IIA.

Following surgery, the dogs were washed with alcohol, bandaged, treated with antibiotics (topical and intravenous) and jacketed to prevent access to the sensor leads and exit

wounds. The animals received daily supportive care and antibiotics for 5 days post-surgery, at which time a 2-5 day program of retraining with the experimental conditions was accomplished. Body temperature, hematocrit, heart rate and volume of pleural effusion were monitored daily. Pressures and flows were recorded daily and catheters were flushed periodically to insure patency. Experimentation was commenced only after all physiological parameters were within normal limits and after the flow probe had thoroughly grown in, as evidenced by a stable baseline flow signal. Care was taken with each animal to minimize post-surgical discomfort and enhance the healing-in of the sensors. The post-operative convalescense prior to commencement of experimentation was usually 8 to 10 days.

B.   Measurements and Instrumentation

1. Measurements. This chronically instrumented, un-anesthetized dog preparation, with respirator mask apparatus was developed to provide direct measurements of the following physiological parameters:

    a.   Circulation

        1.   Heart Rate (HR)

        2.   Stroke Volume (SV)

        3.   Aortic Flow ($\dot{Q}_{AO}$)

      4. Aortic Blood Pressure (P_{AO})

      5. Left Atrial Pressure (P_{LA})

      6. Right Atrial Pressure (P_{RA})

      7. Pulmonary Artery Pressure (P_{PA})

  b. Respiration

      1. Respiratory Frequency (f)

      2. Tidal Volume (V_T)

      3. Minute Volume (V_{min})

      4. Intrapleural Pressure (P_{PL})

      5. Inspiratory Flow ($\dot{V}_I$)

      6. Mouth Pressure (P_{mouth})

Note: Refer to Figure IIB for an example of chart recordings
of the above functions.

  c. Blood Analysis

      1. Oxygen-Hemoglobin Saturation, arterial and
venous (HbO_2)

      2. Hemoglobin (Hb)

      3. pH, arterial and venous

      4. Oxygen Tension, arterial and venous (PO_2)

      5. Carbon Dioxide Tension, arterial and
venous (PCO_2)

      6. Cholinesterase Inhibition, in whole blood
and plasma separately (% Activity)

2. _Derived Values._ From the above directly measured
parameters we have further derived or computed the following:

a. Oxygen consumption (VO_2): the product of cardiac output and difference in arterial-venous oxygen content in ml O_2/ml blood.

b. Transmural circulatory pressures: the instant-aneous difference between a circulatory pressure measured relative to atmospheric pressure minus the intrapleural pressure measured relative to atmospheric at the same instant, i.e., effective pressure across the wall of the vessel or chamber in the thoracic cavity. Data is expressed with the prefix notation TM, as in TMP_{AO}, TMP_{RA}, etc.

c. Inspiratory time relative to the total time of one complete respiratory cycle (T_i/T_{ot}), expressed as a ratio (derived from the $\dot{V}_I$ signal).

d. Shunt Fraction (F_S): the amount of ventilation-perfusion imbalance caused by lung units with low ventilation-perfusion ratios (i.e., regions of alveolar hypoventilation). This value is calculated from the shunt equation in the form:

$$F_S = \frac{\dot{Q}_S}{\dot{Q}_T} = (C_i - C_a)/(C_i - C_v)$$

where $\dot{Q}_S$ is the physiologic shunt and C_i is the O_2 content of ideal end-capillary blood, and C_a and C_v are arterial and venous O_2 contents, respectively.

e. Cardiac Work Rate, left ventricle ($\dot{W}_{LV}$): the external work performed by the left ventricle times heart rate, calculated by the formula:

$$\dot{W}_{LV} = (\overline{TMP}_{ao}, diast. - \overline{TMP}_{LA}) \cdot SV \times HR$$

where $\overline{TMP}_{ao}$, diast is the afterload to left ventricular ejection

in mmHg, $\overline{TMP}_{LA}$ is the mean preload or filling pressure of the ventricle, SV is the stroke volume in ml, and HR is the heart rate. This value represents the mean hydraulic work rate of the left side of the heart expressed in kg·M/minute.

f. An index of the total muscular work rate of inspiring, the Pleural Pressure Time Index (PPTI). This index is derived by extracting the area under the pleural pressure recording from end-expiration to end-inspiration (refer to Appendix B):

$$PPTI = f \cdot \int_{ee}^{ei} P_{PL} \cdot dT_I$$

where f is respiratory frequency, P_{PL} is pleural pressure in mmHg, and T_I is inspiratory time in seconds. The resulting index, therefore, is expressed in units of mmHg and is representative of mean inspiratory pleural pressure, the driving pressure for lung inflation generated by tension development in the inspiratory muscles, displacing the diaphragm downward and the chest wall outward. This index has been shown to correlate well with the oxygen cost of breathing expended by the respiratory muscles (McGregor and Becklake, 1961; Rochester and Bettini, 1976).

g. An index of the effectiveness of the muscular effort of breathing, the Breathing Effectiveness Index (B.E.I.), is defined as follows:

$$B.E.I. = \dot{V}_{min}/PPTI$$

where $\dot{V}_{min}$ is the inspired ventilatory volume in 1 minute

($L \cdot min^{-1}$) and PPTI is defined above. The resulting index, then is expressed in units of $L \cdot min^{-1}$/mmHg. This derived relationship is used to be an expression of the relative efficiency of the ventilatory apparatus to produce inspiratory flow, and it has the units of conductance; thus it is inversely proportional to resistance. Further discussion and interpretation of my development and use of this index is presented in chapter IV.

    3.   Instrumentation/Apparatus.

        a. Circulatory Monitors

An electromagnetic flow transducer (Biotronex, Series 5000), installed around the aortic root, was connected to a pulsed-logic blood flowmeter (Biotronex model BL-610). The flow probes were calibrated in vitro prior to surgical implantation by a gravity-feed system using various flow rates of an ionizable fluid (saline) while timing the collection and measuring the collected volume in a graduate cylinder. This signal was recorded on channel 1 ($\dot{Q}_{ao}$) of a Beckman, type-R, 12-channel dynograph recorder (see Fig. IIB). Zero flow for the aortic flow channel was considered to be the point on the phasic signal occurring just prior to the onset of ejection. Channel 2 on the recorder was the electronically integrated signal from the flow transducer with each heart beat. The height of the signal indicates stroke volume (SV). This recording was calibrated electronically and adjusted to reset with each heart beat. Channel 3 on the

FIG. II B   SAMPLE 12 CHANNEL CHART RECORDING
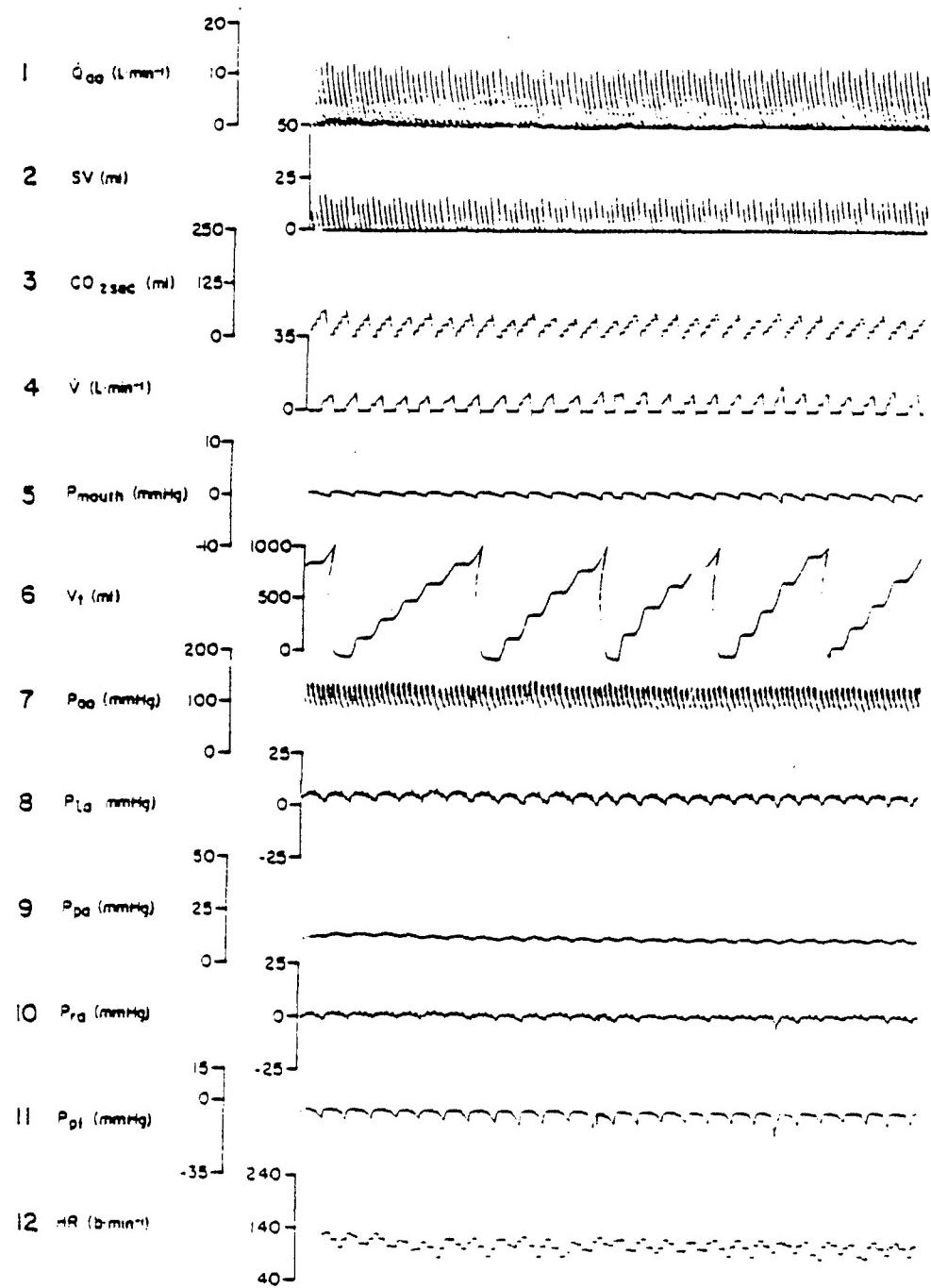
Figure II-B. Sample 12-channel Recording.

Typical simultaneously recorded circulatory and respiratory parameters generated during a control condition. Functions include: (1) aortic flow ($\dot{Q}_{ao}$), (2) stroke volume (SV), (3) cardiac output in 2-second intervals ($CO_{2\ sec}$), (4) inspiratory flow profile ($\dot{V}_I$), (5) mask pressure (P_{mask}), (6) tidal volume integrated from each $\dot{V}_I$ cycle (V_T), (7) aortic pressure (P_{ao}), (8) left atrial pressure (P_{LA}), (9) pulmonary artery pressure (P_{PA}), (10) right atrial pressure (P_{RA}), (11) intrapleural pressure (P_{PL}), (12) heart rate (HR).

recorder was the integrated signal from the flow transducer which was adjusted to reset every two seconds. The height of this signal, therefore, indicated cardiac output during the two-second intervals (CO_2 sec).

All pressure catheters implanted in the dog were connected to Statham P-37 strain gauge pressure transducers, which were connected to the dynograph recorder on various channels. During the experiments all pressure transducers were fixed to a plate attached to the chest wall of the dogs approximating the height of the right atrium. The placement of this plate was indelibly marked on the dog to insure the same positioning throughout subsequent experimental trials. The pleural pressure catheter was air-filled while all other pressure catheters were saline-filled and flushed intermittently throughout each experiment. Phasic aortic pressure (P_{ao}) was recorded on channel 7. Left atrial pressure (P_{LA}), pulmonary artery pressure (P_{PA}), and right atrial pressure (P_{RA}), were recorded on channels 8, 9, and 10, respectively. These signals were electronically filtered to remove unwanted high frequency "noise" (time constants 0.15 sec). Intrapleural pressure (P_{PL}) was recorded on channel 11. The pressure channels were calibrated directly with a syringe and a mercury manometer. The zero pressure level was verified periodically throughout each experiment. Channel 12 recorded instantaneous heart rate (HR) and was derived

electronically from the interval between the onset of two successive ejections into the aorta as recorded by the electro-magnetic flow transducer.

b. Ventilatory Monitors

The dogs were trained to tolerate the wearing of a specially-modified half-face respirator mask which was designed with separate valving for inspiratory and expiratory flows. Inspiratory flow was monitored with a Fleisch pneumotachometer (28 mm diameter) combined with a Valedyne pressure transducer (±2 cm H_2O). A removable round aluminum orifice (6mm I.D.) was inserted into the intake side of the pneumotachometer to introduce the resistance load at appropriate times during the experiments (refer to Figure IIC). The calibration curve showing the nature of the orifice resistor is shown in Figure IID; it illustrates the relationship between flow and pressure drop throughout the physiologic spectrum of flow values. (Resistance = pressure drop/flow). The signal from the Validyne pressure transducer was processed through a Validyne model CD-101 Carrier Demodulator providing a 10 Volt DC signal which was amplified by the Beckman dynograph and recorded on channel 4 as instantaneous inspiratory flow ($\dot{V}_I$). The area under this flow signal was electronically integrated and recorded on channel 6 as tidal volume (V_T). Minute volume (V_{min}) was computed manually as the product of frequency (f) and V_T. A Statham P-37 strain gauge pressure
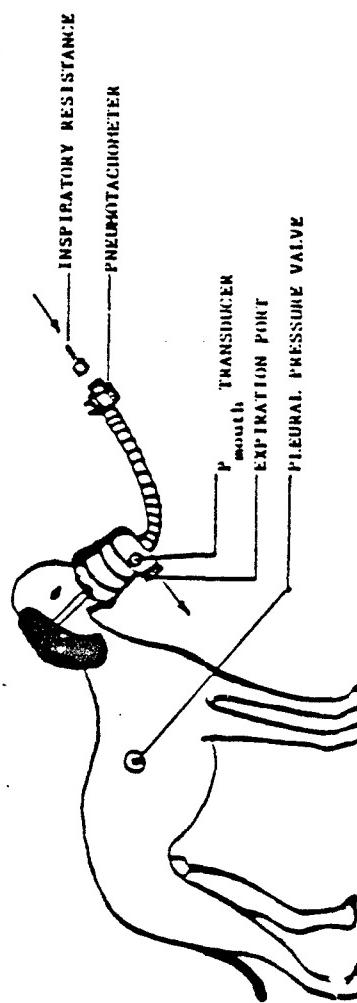
Figure II-C.   Ventilatory Monitors

Schematic representation of the ventilatory pressures and flow monitors _in situ_. The pneumotachometer monitors inspiratory flow only, while pleural pressure and mask pressure are monitored continuously.
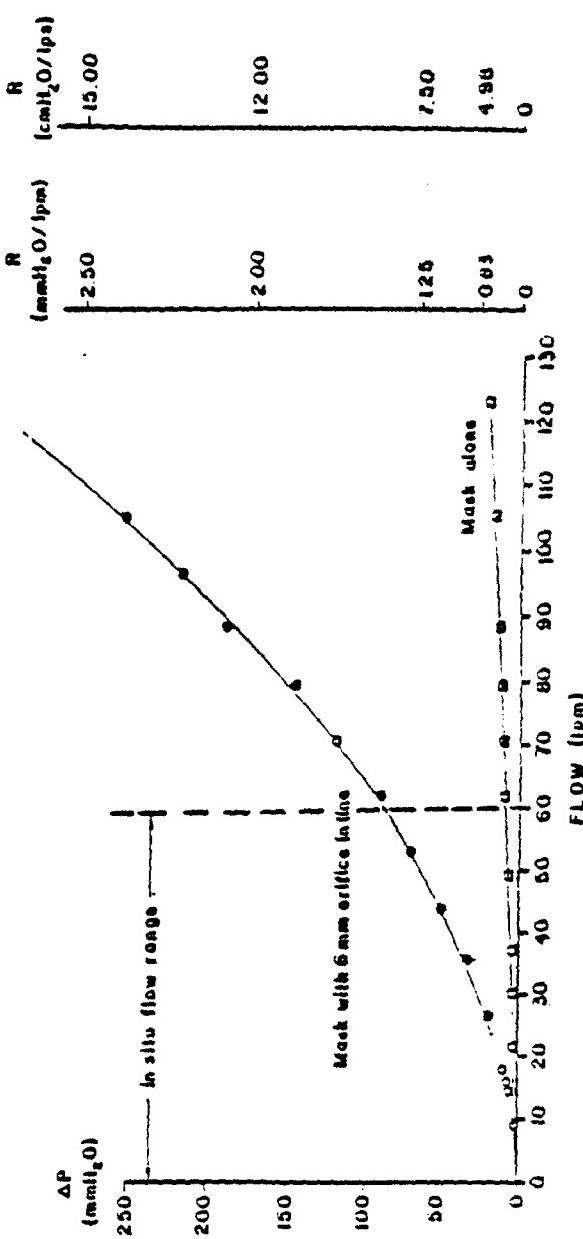
Figure II-D.   Resistance Calibration Curve.

The pressure drop (ΔP) relative to steady-state flow (FLOW) is shown for the respirator mask assembly with and without a 6 mm aluminum orifice resistor in place. Resistance (R) is shown on the right hand scale in units of mmH_2O/lpm and cmH_2O/lps. <u>In situ</u> the maximum flow observed never exceeded 60 lpm.

transducer was attached to a "side on" static pressure orifice in the right side of the respiratory mask and its signal was recorded on channel 5 as mask pressure (P_{mask}).

The pleural pressure valve was connected by a 16-gauge polyvinyl catheter to a Statham P-37 strain gauge pressure transducer; the signal was recorded on channel 11 (P_{PL}).

c. Blood Measurements

Gases. Arterial and venous blood samples were analyzed for PO_2, PCO_2, and pH with a Radiometer BMS MK2 blood gas analyzer (electrode temperature = 37°C). These values were corrected to the normal dog body temperature using the nomogram of Kelman and Nunn (Kelman and Nunn, 1966). Arterial and venous oxygen saturation (HbO_2) and reduced hemoglobin (Hb) were measured with an Instrumentation Laboratory CO-Oximeter Model 182.

Arterial and venous oxygen contents (O_2Ct) were calculated mathematically by multiplying the oxygen capacity of the blood sample (1.39 ml O_2/gram Hb) x Hb expressed in grams x the fraction of oxyhemoglobin expressed as a decimal ($\frac{\% HbO_2}{100}$). The complete expression may be represented by the following equation:

$$O_2Ct = 1.39 \times Hb \times \frac{\% HbO_2}{100} ,$$

where Hb and HbO_2 are expressed to a common base. Data was expressed in terms of milliliters of oxygen at STP per hundred milliliters of blood, or simply, Vol - %.

Oxygen consumption ($\dot{V}O_2$) was computed by multiplying the cardiac output in ml/min by the arterial-venous difference in O_2Ct (ml O_2/ml of blood) rendering O_2 consumption in units of ml O_2/minute.

Cholinesterase activity. A radiometric assay was performed in the laboratory of Dr. T. Guilarte, (Guilarte et al, 1983) to determine plasma and whole blood cholinesterase activity. The radiometric method is based on the hydrolysis of acetylcholine (or other choline esters) by cholinesterase to choline and acetic acid. The H^+ generated from the acetic acid reacts with $NaH^{14}CO_3$, present in the reaction mixture to produce $^{14}CO_2$. The $^{14}CC_2$ produced is easily and accurately quantified using a gas flow system containing an ionization chamber (Bactec R301, Johnston Labs, Cockeysville, MD). The amount of $^{14}CO_2$ measured is proportional to the amount of acetylcholine hydrolyzed. Cholinesterase activity, therefore, can be expressed as umole acetylcholine hydrolyzed/hr/ml plasma (or whole blood), or more simply as a ratio between control activity (100%) and post-neostigmine administration activity. Blood samples were taken at intervals following administration of neostigmine (0.025 mg/kg I.V.) corresponding to the time duration of the dosed phase of the experimental trials. The degree of cholinesterase inhibition induced by 0.025 mg/kg neostigmine followed the time course shown graphically in Figure III; plasma and whole blood activities are shown. The experimental period commenced 15 minutes after
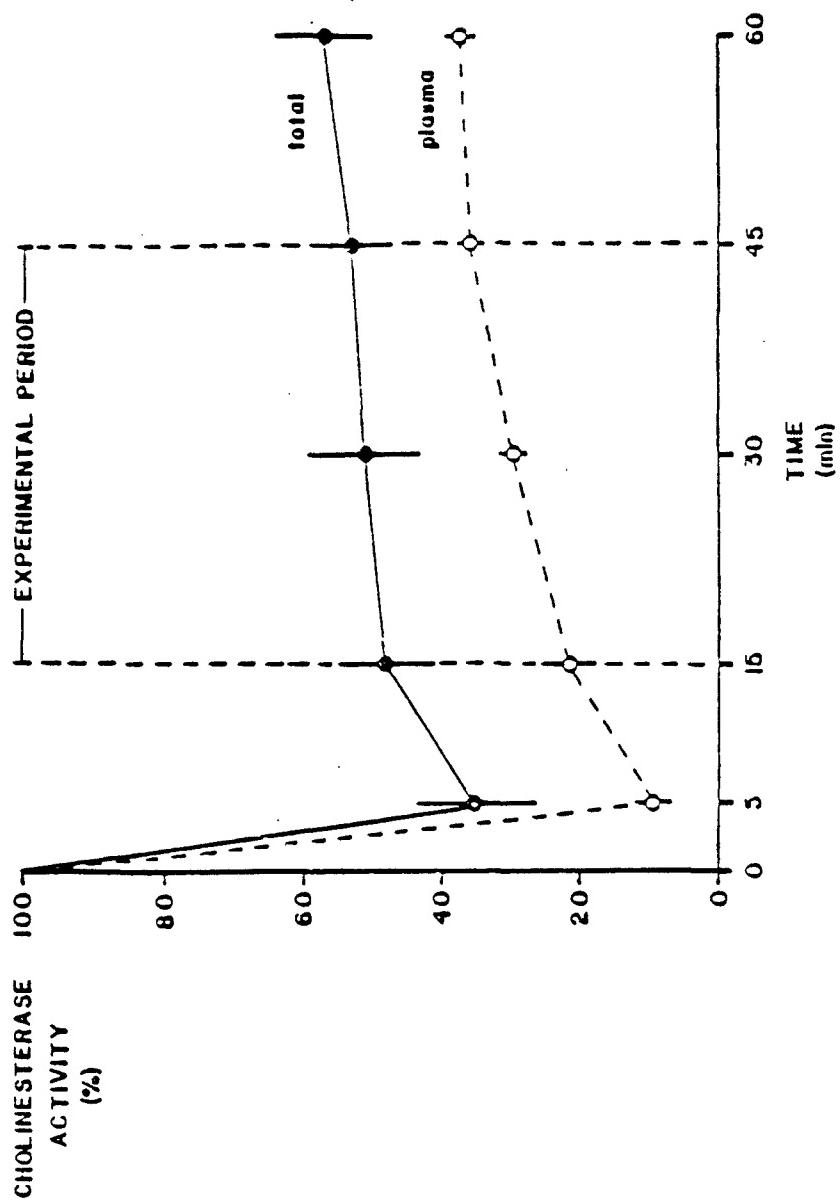
Figure II-E.   Cholinesterase Inhibition Time Course.

The relationship of the neostigmine-induced
inhibition of whole blood (total) cholinesterase and
plasma (butyryl) cholinesterase is shown. Note that the
experimental period begins after the drug has equilibrated
in the bloodstream and continues through a fairly
constant 30 minute period of inhibition.

neostigmine administration and was completed within 30
minutes from that time. Thus, the degree of inhibition
was relatively constant throughout the experimental protocol
without the complication of constant flow drug infusion.
Still, it might be argued that the reactivation curve of
cholinesterase (especially in plasma) is not flat, and thus
the data gathered at the end of the experimental period is
not comparable to that gathered at the beginning, and there-
fore a systematic error is introduced. Indeed this might be
the case, but the protocol consistently placed the exercise
conditions in the latter half of the experimental period and
if any time-related effect is present, it would only serve to
underestimate the inhibition response during exercise. The
resistance loading versus no-resistance data were collected so
close together in time during the neostigmine phase of the
protocol (2 minutes) that this question can be ignored with
reference to this challenge.

Plasma and whole blood activities showed a similar in-
hibition-reactivation relationship with time except that the
mean levels of cholinesterase activity were significantly lower
(30% after 15 min; $p < 0.025$) at each measured point in time.
This disparity was presumed to be due to a greater affinity
of the plasma enzyme (butyryl cholinesterase) for the inhibitor
than is the case for the erythrocyte bound enzyme, acetyl-
cholinesterase. It is of some interest to note that several
authors have suggested that butyryl cholinesterase (plasma ChE)

is the enzyme system that affects the bradycardia response
to anti-ChE agents (Burn and Walker, 1954). It is for this
reason and the uncertain present understanding of the general
significance of this enzyme in other physiological systems
that it's inhibition in this preparation has been monitored.

C. <u>Experimental Design</u>. The objective of this investigation
is to characterize the cardiopulmonary performance effects of
moderate cholinesterase inhibition alone, and in combination
with inspiratory resistance loading, both at rest and during
exercise. Thus, the experimental design embodied three main
treatment variables: 1) state of exercise (E); 2) resistance
to inspiratory flow (R); and 3) moderate cholinesterase
inhibition by adjusted doses of neostigmine (D).

Each of the three treatment variables were studied at
two levels: <u>present,</u> designated by the subscript "$_1$", or
<u>absent</u>, designated by the subscript "$_0$" following the letter
designating the treatment variable (e.g., E_1, E_0; R_1, R_0;
D_1, D_0). The resulting 2 x 2 x 2 factorial design matrix is
presented diagrammatically in Figure IIF; it illustrates the
eight treatment conditions that are the result of forming all
possible combinations of the three main treatment variables at
two levels each (0, 1).

This factorial approach maximized the number of observa-
tions of each treatment variable within a single experimental
design and thereby provides maximal statistical power for

E_0 (rest)

	D_0 (not dosed)	D_1 (dosed)
R_0 (no resistance)	CON	DOS
R_1 (resistance)	RES	R*D

E_1 (exercise)

	D_0 (not dosed)	D_1 (dosed)
R_0 (no resistance)	EXC	E*D
R_1 (resistance)	E*R	E*R*D

Figure II-F.   Experimental Design Matrix.

The 2^3 factorial matrix is diagrammed. Notation
is explained in the text.

interpreting main treatment effects and possible interactions among the treatment variables on the physiological parameters of interest.

D. Experimental Protocol. After the experimental animals had been trained, surgically instrumented, and sufficient healing-in and recovery accomplished, the following experimental routine was observed with each trial:

(1) Calibrated each pressure and flow recording device using appropriate direct methods.

(2) Connected the externalized leads of the surgically implanted sensors on the experimental animal to the amplification and recording system and confirmed their proper baselines and operating ranges.

(3) Fit the respirator mask assembly snugly around the muzzle of the quietly standing dog insuring non-leakage of ventilatory flows. After the animal was calm and breathing quietly (approximately 1 minute), the physiological functions were recorded for three minutes. This was the control condition (CON; that is, E_0, R_0, D_0). At this time, with control conditions maintained, two blood samples were drawn, one mixed venous sample from the pulmonary artery catheter and one arterial sample from the thoracic aorta catheter. These blood samples were placed on ice and held until completion of the experimental trial, at which time they were analyzed.

(4) When the control condition had been completed, the resistance was inserted distal to the inspiratory valve of the mask assembly without visual or audible cues. Recordings were made for a three minute period. This was the inspiratory resistance loading condition (RES; E_0 R_1 D_0). Blood samples were then drawn as above.

(5) The dogs were rested for 5-7 minutes with the mask removed and then the exercise control condition, with the dog running at 2.5 mph on a 10° inclined treadmill, was recorded. This exercise control (EXC; E_1 R_0 D_0) condition was maintained 3 minutes and then blood samples were drawn and held for analysis as above.

(6) As in (4) above, the 6 mm orifice was then inserted and recordings generated for 3 minutes followed by blood samples being drawn. This condition was the exercise and resistance loading combined treatment condition (L * R; E_1 R_1 D_0).

(7) The dogs were rested for 30 minutes in place and given water.

(8) After the rest period, neostigmine was administered (0.025 mg/kg body weight) intravenously through the right atrial catheter. After a 15 minute wait for equilibration of drug-induced symptomatology, steps (1) through (6) were repeated as above. The resulting treatment conditions were labeled DOS (E_0 R_0 D_1), R * D (E_0 R_1 D_1), E * D (E_1 R_0 D_1), and E * R * D (E_1 R_1 D_1), respectively.

At the conclusion of this experimental routine, the dogs were watered and fed and all calibrations and zeros verified. Blood samples were immediately analyzed for gas tensions and cholinesterase activity.

E. Discussion of Methodology. The value of an unanesthetized animal preparation capable of monitoring and recording cardiopulmonary parameters cannot be overstated, especially when trying to draw inferences about integrated autonomic reflex control mechanisms of cardiopulmonary functions. However, the very nature of this type of experimental preparation brings with it certain compromises of control in the experimental protocol. Great efforts were made to insure constancy of the laboratory setting in these experiments and the subject animals were trained to similar degrees with similar techniques and were matched for weight and approximate age. Control measurements were often substantially variable between animals and even from day to day within the same animal. However, the analysis of variance procedures used in handling the data and drawing inferences concerning significance were unbiased and allow conclusions to be drawn on treatment effects and interactions between combined treatments.

The selection of level of treadmill exercise for this study (3mph, 10° incline) was based more on the pragmatic concerns of what the animals were "willing" to tolerate rather

than on specific physiological criteria (e.g., % of $\dot{V}O_2$max), and as a result the conclusions drawn from the exercise data may be "premature" in their application to more rigorous exercise bouts. On the other hand, one might argue that measureable changes in cardiopulmonary functions at low exercise challenge will likely be more pronounced at the more severe performance levels.

The question of steady-state also comes up in this experimental protocol. Most treatment challenge phases were only three minutes in duration, with data generated during the final 30 seconds of each phase. In this case, the low level of exercise argues favorably for the achievement of a steady-state response since a mild exercise challenge takes less time to "adjust" cardiopulmonary functions (e.g., heart rate, cardiac output, ventilation, etc.). On the other hand, tendencies toward muscular fatigue and possible oxygen debt situations were doubtlessly curtailed before they developed fully. In any case, all experiments were handled with an identical protocol and interrupted runs were discarded, thus allowing valid treatment effect comparisons even if the last small adjustments to steady-state had not been captured by the data collection.

Another matter of methodology requires discussion: The use of the PPTI (Pleural Pressure-Time Index) to characterize the oxygen cost of breathing (O_2CB). Preservation of respiratory gas exchange in situations which increase the

work of breathing (e.g., in these experiments: inspiratory
resistance loading; cholinergic broncho-constriction,
exercise) depends on the ability of the respiratory muscles
to sustain high levels of effort for prolonged periods.
The determinants of respiratory muscle endurance under
these load conditions are unclear. When limb skeletal
muscles perform rhythmic work, their ability to sustain
effort appears to depend on muscle blood flow, since at very
high work rates blood flow fails to increase in proportion
to energy expenditure and fatigue ensues (Mottram 1958;
Monod and Scherrer 1965). Tenney and Reese (1968) have
postulated that these same considerations hold for the
respiratory muscles. Rochester and Bettini (1976), measuring
diaphragmatic blood flow and diaphragmatic oxygen consumption
under conditions of increasing inspiratory resistance loads,
showed that the diaphragm's oxygen requirements are met by
an increase in blood flow and an increase in oxygen extraction.
In this regard the diaphragm resembles other skeletal muscles
(Mottram 1958). However, oxygen extraction tends to plateau
at the higher loads while diaphragmatic blood flow continues
to rise; thus, like the heart, the diaphragm depends on per-
fusion to meet its oxygen needs. Rochester and Bettini further
showed excellent correlation of diaphragmatic oxygen consumption
(O_2C3 diaphragm) with an index of inspiratory muscle tension,
the Pleural Pressure-Time Index (PPTI). The PPTI was adapted
for use in this study because it reflects muscular "work" of

breathing much better than the traditional external work
of breathing methods of Campbell (1970) and Goldman-Grimby-
Mead (1976). These external work of breathing methods
utilize the usual concepts of pressure-volume relation-
ships to quantify work, and to this end they are of little
use in predicting "muscle work" (that is, oxygen consumption
of the inspiratory muscles) unless the efficiency of the
ventilatory muscular mechanism is known:

$$\text{Efficiency} = \frac{\text{Work of Breathing}}{O_2 \text{ Cost of Breathing}}$$

This ratio has proven to be a factor with very wide variation
ranging from 1 to 25% (Campbell et al, 1957; 1959), and further,
it changes with the pattern of breathing, with inspiratory
loading, and with the level of functional residual capacity
(i.e., lung volume at end-expiration). Consequently, external
work of breathing would be a very poor predictor of "muscle
work" in the present experiments since all these factors are
subject to change in response to the experimental challenge
conditions.

The PPTI, on the other hand, has been shown to correlate
well with inspiratory muscles, oxygen consumption, and it is
sensitive to changes in loading and pattern and frequency of
breathing (Rochester and Bettini, 1976; Robertson et al, 1977
a,b,). Unlike the external work calculations, the PPTI in-
cludes not only the tension developed by the inspiratory
muscles, but also the duration of the contraction. Thus it
correlates well with inspiratory muscle oxygen consumption,

and when used as the denominator of the ratio formed from
minute ventilation/PPTI, an index of effectiveness of the
inspiratory muscles in producing air flow is obtained.
This is the Breathing Effectiveness Index (BEI) and it has
the units of conductance (L · min^{-1}/mmHg). It should be
emphasized here that this index is _not_ airway conductance
(of its reciprocal, resistance) since it incorporates factors
of not only flow resistance but also forces required to
overcome elastic recoil of the lung and chest wall, and
other tissue factors. However, if we assume that these
tissue factors have not changed in our experiments, then
the BEI can be used to represent changes in inspiratory
muscle efficiency during inspiration.

F.   _Data Analysis and Statistics._ All data were analyzed
with an analysis of variance procedure incorporating a mixed
effects model with the three main treatment variables con-
sidered fixed, and variation between dogs considered random
(so-called block effects). The dogs (blocks) were assumed
to be representative of a larger population and inferences
are made for the population of mongrel dogs in general. The
replication error (three replications of each experimental
trial for each of the five dogs in the study) is considered
random and is used as the error term for many of the hypo-
theses concerning treatment Main Effects and interactions.
However, in the cases where the Dog x Treatment interactions

were larger than the replication error mean square (E.M.S.),
that interaction E.M.S. was used as the error term to test
that particular main effect (Steele, and Torrie, 1960).

When significant interactions were shown with the
above analysis of variance for individual physiological
parameters the simple treatment effects were tested for
significant differences with Duncan's New Multiple Range
test. (Duncan D.B., 1955).

Each circulatory and respiratory parameter under study
was manually extracted from the 12-channel recordings that
were accumulated during the experimental protocol for each
trial. Twenty-second periods were counted and meaned by
hand and these values were used as the raw data for com-
pilation and subsequent analysis. Blood gas values were
derived by averaging two replicates corresponding to each
treatment condition from each experimental trial, and then
subjected to the analysis of variance procedure as above.
Data is presented graphically in this text summarizing across
dogs and replications with mean data compiled for each of the
eight (2^3) factorially designed treatment conditions. However,
main treatment effects and interactions are also presented
and interpreted as the "average" effect of a treatment
variable.

Note: Main effects and interactions are defined here in the
statistical sense. A treatment main effect, measured on a
particular outcome variable (i.e., frequency, heart rate, etc.),

is constructed by comparing all observations of the outcome
variable when the treatment is present with all the obser-
vations of that same variable when the treatment is
absent and then testing that difference with an appropriate
F ratio for significance. Main effects are not directly
interpretable, however, when significant interaction is
present. Again, the term interaction is used in the
statistical sense. Briefly, in these experiments, it is
a measure of the reproducibility of a response to a treat-
ment variable under one condition compared to the response
to that same variable under another condition. For example,
inspiratory resistance loading without cholinesterase in-
hibition prolonged inspiratory time significantly; by con-
trast, during cholinesterase inhibition, the addition of
inspiratory resistance loading had no effect on inspiratory
time. Thus, an interaction of resistance with cholinesterase
inhibition (R*D) exists in this example. Other pertinent
interactions that have been tested for each outcome variable
include resistance with exercise (E*R) and cholinesterase
inhibition with exercise (E*D). Significant three-factor
interactions were not observed in this study.

CHAPTER III.   RESULTS OF EXPERIMENTS

Results are presented in the form of mean values from five experimental animals. Four of the five dogs completed three replications of the entire experimental protocol and one dog completed two full replications. The resulting means, therefore, were derived from fourteen separate experimental days. In those instances, where there were exceptional differences between dog responses to a particular experimental treatment challenge, special footnotes or comments are included. Physiologic response parameters have been grouped into three main categories: 1) ventilatory functions, 2) cardiovascular functions, and 3) blood gases, and are presented in that order.

A.   Ventilatory Functions at Rest and Exercise (Table IIIA and Figures IIIA, B, C).

1.   Inspiratory Resistance Loading

a) Inspiratory resistance loading at rest (the RES condition) decreased respiratory frequency slightly (1.4 breaths/min) and decreased tidal volume by 20 ml, thereby diminishing minute volume from a control level of 12.48 L/min down to 11.27 L/min, a decrease of approximately 11%.

RES prolonged inspiratory time (expressed as a ratio relative to total time of complete respiratory cycle, T_i/T_{tot})

TABLE III A. VENTILATORY FUNCTIONAL PARAMETERS, SIMPLE EFFECTS

PARAMETER	ABBREV.	UNITS	TREATMENT CONDITION							
			CON	RES	DOS	R*D	EXC	E*R	E*D	E*R*D
Respiratory Frequency	f	(breaths/min)	40.8±5.1	39.4±4.4	55.8±7.5	53.2±7.1	52.7±4.6	49.9±4.7	61.0±5.8	55.5±5.6
Tidal Volume	V_t	(ml)	306±18.9	286±18.1	275±23.4	252±24.4	419±26.4	385±22.0	342±13.1	314±19.4
Minute Volume	V_{min}	(l/min)	12.48±1.27	11.27±0.99	15.35±1.33	13.41±0.74	22.08±1.51	19.21±1.15	20.86±1.39	18.54±1.13
Inspiratory Time/Total Time	T_i/I_{tot}		.46±.01	.49±.01	.47±.01	.48±.01	.50±.01	.53±.01	.50±.01	.51±.01
Inspiratory Peak Flow	V_{peak}	(l/min)	32.6±3.6	27.8±2.15	37.0±2.7	29.2±1.6	56.3±4.3	41.4±2.6	53.7±3.7	42.2±2.3
Mask Pressure	P_{mask}	(mmHg)	-.42±.03	-1.60±.13	-.54±.07	-1.81±.13	-.72±.11	-2.82±.24	-.86±.09	-2.94±.21
Peak Inspiratory Pleural Pressure	PIP_{pl}	(mmHg)	-9.6±0.5	-11.2±0.4	-14.2±2.2	-14.5±1.7	-15.6±0.9	-19.1±0.9	-19.4±1.7	-22.4±1.6
Peak Expiratory Pleural Pressure	PEP_{pl}	(mmHg)	-4.4±0.3	04.3±0.4	-3.3±0.5	-4.0±0.5	-1.0±0.5	-0.9±0.4	+0.6±0.6	+0.6±0.7
Pleural Pressure-Time Index	PPTI	(mmHg)	1.45±.12	1.96±.19	2.96±1.26	2.82±.74	3.6±.39	5.3±.58	5.6±1.5	6.59±.68
Breathing Effectiveness Index	BEI	$\left(\frac{l \cdot min^{-1}}{mmHg}\right)$	8.6±1.57	5.75±.78	5.19±2.11	4.76±1.07	6.02±.97	3.62±.43	3.5±.90	2.81±.55

REST ———————————————————— EXERCISE ————————————————————

from .46 to .49. Peak inspiratory flow was decreased by approximately 5 L/min. Peak inspiratory mask pressure fell by 1.2 mmHg (1.63 cmH_2O), peak inspiratory pleural pressure fell by 1.6 mmHg (2.18 cmH_2O); and end-expiratory pleural pressure remained unchanged at approximately -4.4 mmHg, indicating that functional residual capacity had not changed in response to the load.

The Pleural Pressure-Time Index (PPTI), which indexes the muscular work rate of breathing rose by 35%. The breathing effectiveness index, which is an expression of relative change in efficiency of the inspiratory muscles to produce ventilatory flows, fell by 33% due to the resistance load.

b) Inspiratory resistance loading during fixed treadmill exercise (the E*R treatment condition) produced a slight decrease in respiratory frequency (3 breaths/min), and tidal volume was diminished by 34 ml (8%) compared to the exercise control condition (EXC). The resulting minute volume was therefore diminished 2.87 L/min (13%), similar to the resistance loading effect at rest.

E*R increase T_i/T_{tot} from .50 to .53 and decreased inspiratory peak flow by nearly 15 L/min (26%), reflecting the increased impedance to inspiration caused by the mask resistor. Peak inspiratory mask pressure dropped by 2.10 mmHg as a result of the external resistance of the mask, and peak inspiratory pleural pressure fell to -19.1 mmHg, a drop from

exercise control of 22%. End-expiratory pleural pressure again remained unchanged.

The PPTI increased ~ 45%, while the BEI fell by 40%, compared to the exercise control (EXC).

2. Cholinesterase Inhibition

a) Inhibition of whole blood cholinesterase by 50% (dosage of 0.025 mg/kg neostigmine, I.V.) at rest (the DOS condition) increased respiratory frequency by 15 breaths/min (37%) and decreased tidal volume by 31 ml (10%), resulting in an overall increased minute volume of 31% (from a rest control level of 12.48 L/min, to 15.35 L/min after inhibition).

DOS produced a slightly elongated inspiratory phase of the respiratory cycle relative to expiration (T_i/T_{tot} increased % from .46 to .47) and inspiratory peak flow increased by 4.4 L/min (13.5%). Peak inspiratory mask pressure rose very slightly (0.12 mmHg) concomitant with the increased peak flow. Peak inspiratory pleural pressure fell by -4.6 mmHg, whereas end-expiratory pleural pressure remained unchanged.

The PPTI rose by 104% , and the BEI fell by 40%, compared to control.

b) Cholinesterase inhibition during fixed treadmill exercise (the E*D condition) increased respiratory frequency by 8.3 breaths/min (16%) and decreased tidal volume by 77 ml (18%) resulting in a 6% decrease in minute volume (1.22 L/min). The frequency and tidal volume response is consistent with

the ventilatory response at rest in direction but not magnitude with the result that minute volume is not maintained.

E*D had no measureable effect on T_i/T_{tot} but peak inspiratory flow was lowered by 2.5 L/min (5%) compared to exercise control. Peak inspiratory mask pressure was essentially unchanged, while peak inspiratory pleural pressure fell to -19.4 mmHg and end-expiratory pleural pressure remained at the control level.

The PPTI rose by 55%, while the BEI fell 39% as a result of cholinesterase inhibition during exercise.

3. Inspiratory Resistance Loading Combined with Cholinesterase Inhibition.

a) The combined challenge of inspiratory resistance loading while the dogs were under cholinesterase inhibition, at rest (the R*D condition), increased respiratory frequency by 12.4 breaths/min (30%) and decreased tidal volume by 54 ml (18%). The resulting minute volume reflected a net increase of 1 L/min (8%) above control values and is intermediate between the response to resistance loading and the response to cholinesterase inhibition acting separately.

R*D increased T_i/T_{tot} from .46 to .48 and decreased peak inspiratory flow by 3.4 L/min (10.5%). Peak inspiratory mask pressure fell by 1.4 mmHg secondary to the external mask resistance and peak inspiratory flow. Peak inspiratory pleural pressure dropped to -14.5 mmHg, which was the lowest peak

FIGURE III A

FREQUENCY 60
(resp /min)

A

TIDAL
VOLUME
(ml)

B

MINUTE
VOLUME
(L/min)

C
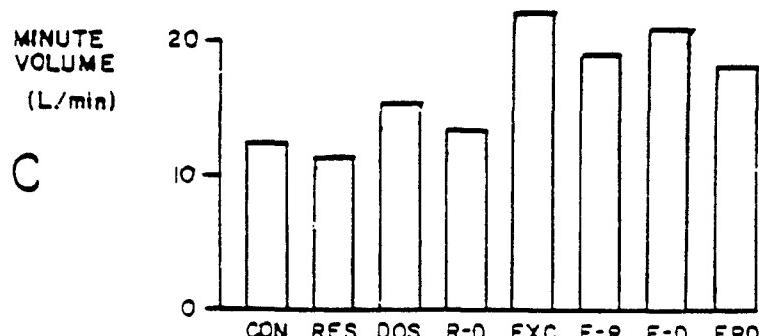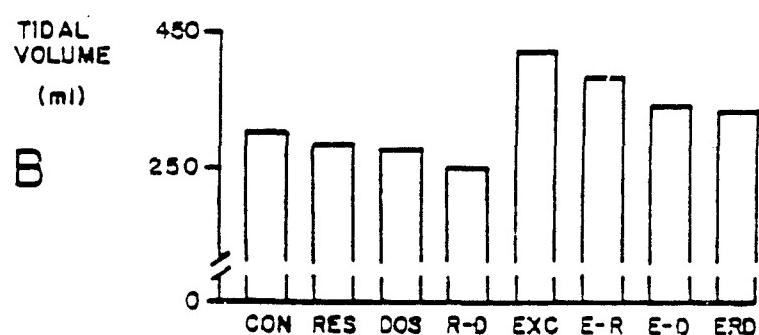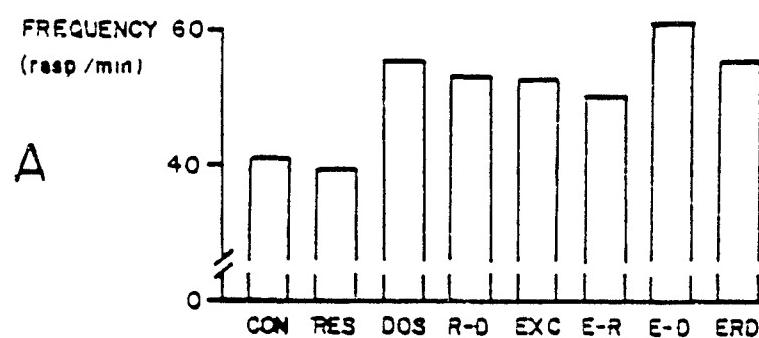
Figure III-A.   Alterations in Ventilatory Parameters,

Simple Effects.

Panel A graphically depicts the changes in respiratory

frequency through each of the eight challenge conditions in

the experimental protocol (refer to Chapter II, paragraph

D. Experimental Protocol for a complete explanation of

the notation used). Panel B depicts the changes in tidal

volume, and Panel C depicts the changes in minute volume.

Note that the first four bars in each panel represent the

at rest conditions while the latter four bars represent

the exercise conditions. CON is the rest control condition;

EXC is the exercise control condition. RES, DOS, and R-D

refer to the resistance load condition, the neostigmine

dose condition, and resistance combined with dose condition,

respectively. The same pattern continues for the exercise

conditions; that is, E-R, E-D, and ERD refer to exercise with

resistance, exercise with dose, and exercise with resistance

combined with dose.

Note: The above notation for the experimental conditions

holds for all of the Simple Effects bar graphs presented (eight bars).

FIGURE III A'

FREQUENCY 60
(resp./min)

A

TIDAL
VOLUME
(ml)

B

MINUTE
VOLUME
(L/min)

C
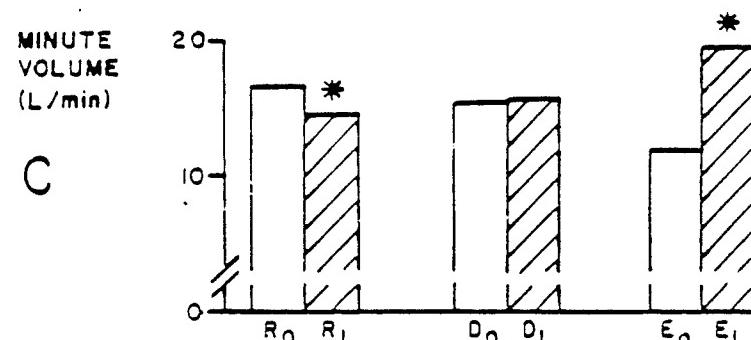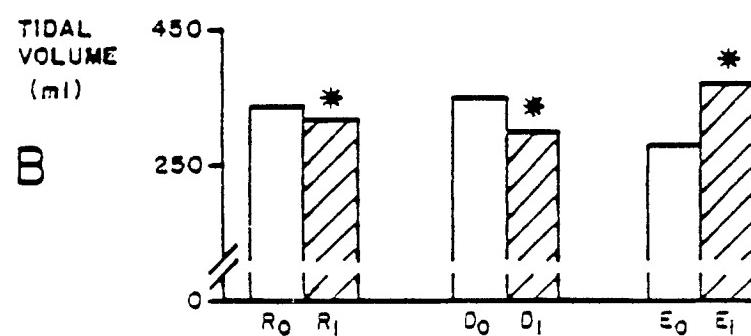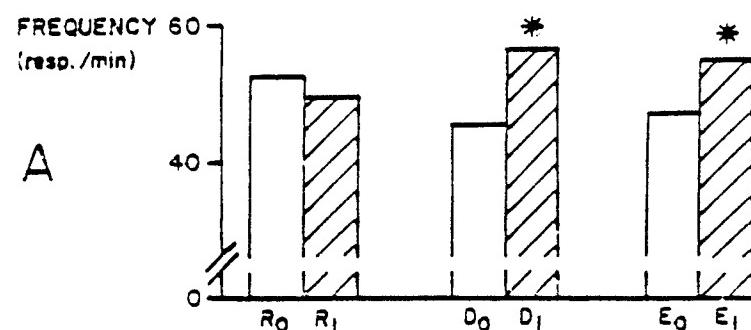
Figure III-A'. Alterations in Ventilatory Parameters,

Treatment Main Effects.

Panel A depicts the main effects of each of the three
main treatment variables, resistance (R_0 vs R_1), dose
(D_0 vs D_1), and exercise (E_0 vs E_1), on respiratory frequency.
The cross-hatched bars depict the "treatment present" mean
data, while the open bars represent the "treatment absent"
mean data for contrast. The difference in the two values
is thus the "treatment main effect". An asterisk (*) on the
cross-hatched bar denotes a statistically significant main
effect (p < .05).

Note: This notation scheme holds for all of the Main Effects
bar graphs presented (6 bars). Panel B presents the treatment
main effects of resistance, cholinesterase inhibition, and
exercise on tidal volume (V_T). Panel C presents the treatment
main effects on minute volume (V_{min}). Note that there is no
significant main effect of cholinesterase inhibition (D_1) on
this parameters, but the analysis of variance uncovered a
significant interaction between cholinesterase inhibition and
exercise (see Table IIIA' and text).

FIGURE III B

A — PLEURAL and MASK PRESSURE (mmHg)

* = P_{mask}
□ = P_{pl}

CON RES DOS R-O EXC E-R E-O ERO

B — PEAK FLOW, INSPIRATION (L/min)

CON RES DOS R-O EXC T-R E-O ERO

C
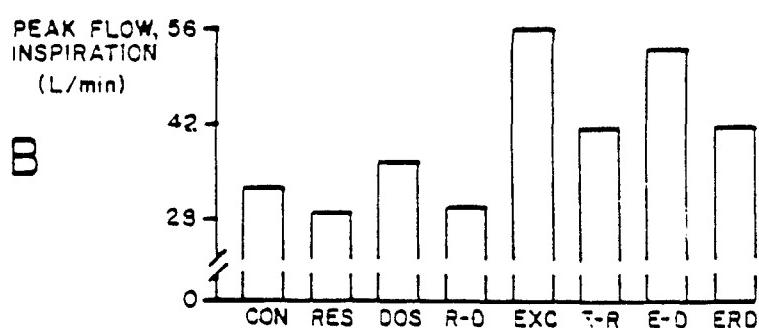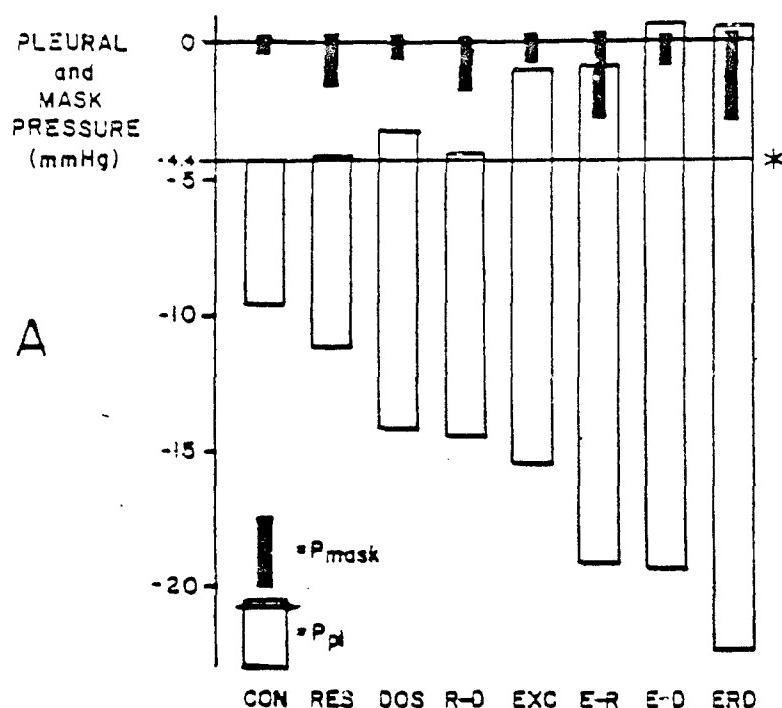
Figure III-3.   Alterations in Ventilatory Pressures and

Flows, Simple Effects.

Panel A graphically presents the oscillatory pleural

and mask pressures generated by the factorial protocol.

The solid narrow bars represent mask pressure (P_{mask}) and

the open wide bars represent the oscillatory pleural

pressures, with the lowermost boundary of each bar showing

peak inspiratory pressures and the uppermost boundary

showing peak expiratory pressures.   Panel B graphically

presents the changes in peak inspiratory flow throughout

the protocol.   Notice the consistent depression of peak flow

in the resistance loaded conditions (RES, R-D, E-R, ERD).

FIGURE III B'

PLEURAL
and
MASK
PRESSURE
(mmHg)

A

R_0 R_1 D_0 D_1 E_0 E_1

PEAK FLOW, 56
INSPIRATION
(L/min)

B

R_0 R_1 D_0 D_1 E_0 E_1
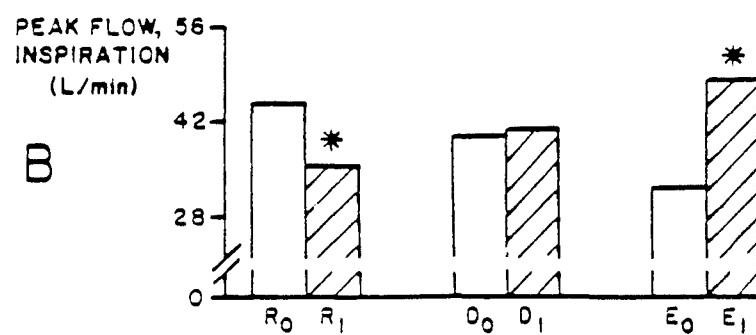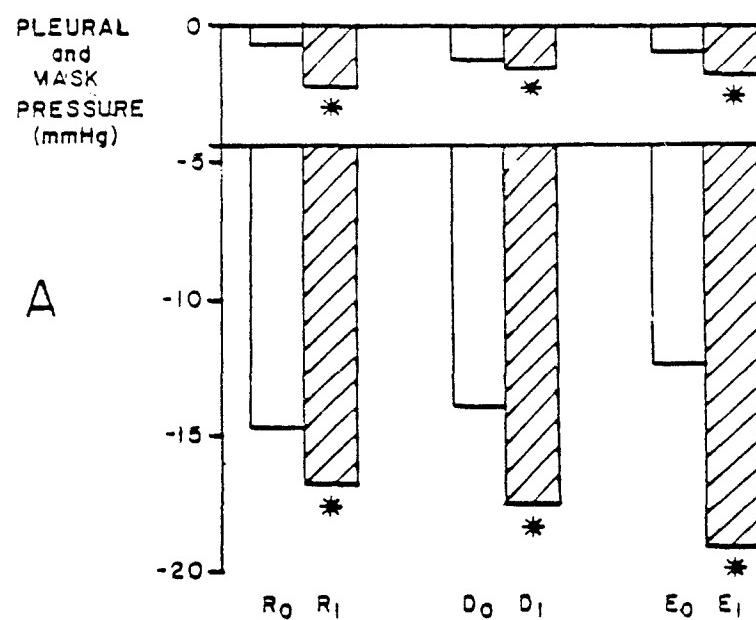
Figure III-B'. Alterations in Ventilatory Pressures and

Flows, Treatment Main Effects.

Panel A depicts the treatment main effects on mask and

pleural pressure. Note that the pressure drop in the mask

(P_{mask}) from resistance loading is large and not related to

an increased peak flow (panel B), whereas the dose and

exercise main effects are associated with increased flows.

Similarly for pleural pressure (the longer bars in panel A),

the increase in pressure drop required of the inspiratory

muscles to generate flow in the resistance-loaded conditions

is not related to increased flow ($\dot{V}_{peak}$ falls significantly)

but rather directly reflects the increase in external resistance

imposed by the mask's orifice resistor. Conversely, the

pleural pressure drop seen in the dosed (cholinesterase

inhibited) condition is not associated with an increased

external resistance but, rather is a function of cholinergically

stimulated bronchoconstriction and/or decreased lung compliance,

probably both.

FIGURE Ⅲ C

PLEURAL
PRESSURE -
TIME INDEX
(mmHg)

A

BREATHING
EFFECTIVENESS
INDEX
(L·min⁻¹/mmHg)

B

Figure III-C.   Alterations in Pleural Pressure Time
               Index nd Breathing Effectiveness Index,
               Simple Effects.

     Panel A shows the progressive and incrementally
additive nature of the challenge conditions on this index
of the muscular work of breathing (PPTI). The only
inconsistency in this trend is shown in the DOS
condition, where the PPTI of a single "hyper-reactive"
dog skews the data upward in this cholinesterase in-
hibited condition (the pulmonary mechanics data of
the "hyper-reactive" dog are presented separately
in Table IV-A, and discussed in Chapter IV). Panel B
presents the alterations in "efficiency" of the
respiratory pump as indexed by the BEI. Note the
consistent and approximately additive effects of
each of the challenge loads on breathing effectiveness.

FIGURE III C'

PLEURAL
PRESSURE-
TIME INDEX
(mmHg)

A

C

BREATHING
EFFECTIVENESS
INDEX
(L·min⁻¹/mmHg)

B

Figure III-C'.   Alterations in Pleural Pressure Time

Index and Breathing Effectiveness

Index, Treatment Main Effects.

Panel A depicts the treatment main effects on the PPTI.
Each treatment variable produces a significant demand on the
inspiratory muscles, with exercise producing the most marked
effect of the three loads. Panel B presents the treatment
main effects on the BEI. All three main treatment variables
diminish the efficiency of the respiratory pump significantly.

pressure recorded in these experiments during the rest phase of the protocol. End-expiratory pleural pressure remained at the control level.

The PPTI rose by 94%, while the BEI fell 45%.

b) Resistance loading combined with cholinesterase inhibition during exercise (the E*R*D condition) showed a moderately increased respiratory frequency (2.3 breaths/min; 5%), a decreased tidal volume (85 ml; 20%), and an overall decrease in minute volume of 3.54 L/min (16%).

E*R*D produced a slightly increased T_i/T_{tot} (.50 to .51), and diminished inspiratory peak flow by 14.1 L/min (25%). Peak inspiratory mask pressure dropped by 2.22 mmHg, corresponding to the calibrated resistance and flow of the mask configuration. Peak inspiratory pleural pressure plunged to -22.4 mmHg, which was the lowest mean level recorded during exercise in these experiments. End-expiratory pleural pressure was maintained at the control level during this condition and throughout the entire protocol (-4.4 mmHg).

The muscular work of breathing (PPTI) rose by 80% and breathing effectiveness (BEI) fell 53% compared to exercise control values (EXC).

4. <u>Main Effects and Interactions of Treatment Variables on Ventilatory Functions</u> (refer to Table IIIA[1] and Figures III A[1], B[1], C[1]).

TABLE III A'. VENTILATORY FUNCTIONAL PARAMETERS, MAIN TREATMENT EFFECTS

PARAMETER	ABBREV.	UNITS	Resistance(R)		CHE Inhibition(D)		Exercise(E)		Interactions (see footnotes)
			R_0	R_1	D_0	D_1	E_0	E_1	
Respiratory Frequency	f	(breaths/min)	52.6 NS	49.5	45.7 *	56.4	47.3 *	54.8	None
Tidal Volume	V_t	(ml)	336 *	314	345 *	301	280 *	370	None
Minute Volume	V_{min}	(L/min)	16.6 *	14.5	15.5 NS	15.6	11.9 *	19.2	E x D [1]
Inspiratory Time/Total Time	TI/T_{tot}		.482 *	.502	.495 NS	.489	.474 *	.509	None
Inspiratory Peak Flow	$\dot{V}_{peak}$	(l/min)	44.6 *	35.4	39.7 NS	40.3	32.1 *	48.0	E x R [2]
Mask Pressure	P_{mask}	(mmHg)	-0.64 *	-2.26	-1.37 *	-1.53	-1.08 *	-1.82	E x R [3]
Peak Inspiratory Pleural	PIP_{pl}	(mmHg)	-14.7 *	-16.8	-13.9 *	-17.6	-12.4 *	-19.1	E x R [4]
Peak Expiratory Pleural Pressure	PEP_{pl}	(mmHg)	-2.0 NS	-2.2	-2.7 NS	-1.5	-4.0 *	-0.2	None
Pleural Pressure-Time Index	PPTI	(mmHg)	3.44 *	4.17	3.09 *	4.51	2.30 *	5.31	None
Breathing Effectiveness Index	BEI	$(l \cdot min^{-1}/mmHg)$	5.87 *	4.24	6.00 *	4.11	6.08 *	4.03	None

(1) V_{min}
at rest, E_0: D_0 11.5 * D_1 14.4
at exercise, E_1: 20.6 NS 19.7

(2) V_{peak}
at rest, E_0: R_0 34.6 * R_1 29.5
at exercise, E_1: 54.6 * 41.3

(3) F_{mask},
rest versus exercise not presented since the interaction merely shows the non-linear nature of the resistance orifice.

(4) PIP_{pl},
at rest, E_0: R_0 -11.9 NS R_1 -12.9
at exercise,
E_1: -17.4 * -20.8

a) Respiratory Frequency (f), refer to Figure

III A', panel A)

(1) The computed main effect of inspiratory resistance loading on F was a significant decrease from 52.6 breaths/min to 49.5 breaths/min. This effect is not statistically significant (N.S.) in magnitude, but is consistent, in direction at least, to findings of other investigators (James 1977).

(2) The computed main effect of moderat (50%) cholinesterase inhibtion on F was a significant increase from 45.7 breaths/min to 56.4 breaths/min (p < .05).

(3) The computed main effect of exercise of f was a significant increase from 47.3 at rest to 54.8 during exercise (p < .01).

(4) There were no significant interactions among the three main treatment variables on respiratory frequency, therefore the main effects may be interpreted directly as applying across all treatments combinations.

b) Tidal Volume (V_T, refer to figure IIIA', panel B).

(1) The computed main effect of inspiratory resistance loading on V_T was a significant decrease from 336 ml to 314 ml (p < .03). This finding is in contrast to the typical human response, where resistance loads generally reduce frequency and increase tidal volume. In these animals under these experimental conditions that response was not observed.

(2) The computed main effect of cholinesterase inhibition on V_T was a decrease from 349 ml to 301 ml (p < .05). However, frequency had been increased by an amount sufficient to hold minute ventilation unchanged overall (see below).

(3) The computed main effect of exercise on V_T was an increase from 280 ml at rest to 370 ml during exercise (p < .01).

(4) There were no significant interactions among the three main treatment variables on tidal volume.

c) Minute Volume (V_{min}, Figure III A[1], panel C).

(1) The computed main effect of inspiratory resistance loading on V_{min} was a significant decrease from 16.6 L/min to 14.5 L/min (p < .03), a product of diminished frequency and tidal volume.

(2) The computed main effect of cholinesterase inhibition on V_{min} was negligible (N.S.) that is the increased frequency was offset by a decrease in tidal volume just adequate to maintain minute ventilation unchanged.

(3) The computed main effect of exercise on V_{min} was an increase from 11.9 L/min at rest to 19.22/min during exercise (p < .05).

(4) There were no statistically significant interactions among the three main treatment variables on minute volume, however, the separate analyses of cholinesterase inhibition on V_{min} at rest and during exercise showed small but

opposite results. At rest, the inhibition resulted in an
increase from 11.3 L/min before neostigmine to 12.5 L/min
after dosing. During exercise the inhibition decreased
V_{min} from 19.7 L/min to 18.6 L/min. These differences just
failed to reach the 5% level of significance by Duncan's
test.

d) Inspiratory Time Ratio (T_i/T_{tot})

(1) The computed main effect of inspiratory re-
sistance loading on T_i/T_{tot} was a significant but small
increase from 0.48 to 0.50 (p < .05). This small difference
in inspiratory time ratio is statistically significant owing
to the remarkably small range of values among this group of
experimental animals, and the consistency of the direction of
change.

(2) The computed main effect of cholinesterase
inhibition on T_i/T_{tot} was negligible (N.S.), that is even
though frequency was significantly increased during inhibition
and concomitantly T_{tot} decreased, the relationship of time
required for inspiration relative to total time had not changed.

(3) The computed main effect of exercise on T_i/T_{tot}
was an increase from .47 at rest to .51 during exercise (p < .05).

(4) There were no significant interactions among
the three main treatment variables on inspiratory time ratio.

e) Peak Inspiratory Flow ($\dot{V}_{peak}$, Figure III 3[1], panel 3).

(1) The computed main effect of inspiratory resistance
loading, was to decrease $\dot{V}_{peak}$ from 44.6 L/min to 35.4 L/min (p<.02).

In conjunction with an increased T_i/T_{tot}, this reduction in peak flow had the effect of lengthening and flattening the inspiratory flow profile.

(2) The computed main effect of cholinesterase inhibition on $\dot{V}_{peak}$ overall was a very slight and insignificant increase (0.66 L/min, N.S.). However, similar to the response of $\dot{V}_{min}$ to cholinesterase inhibition, there was an opposite response of peak flow at rest compared to exercise; a) at rest $\dot{V}_{peak}$ increased from 30.7 to 33.4 L/min (N.S.); b) during exercise, $\dot{V}_{peak}$ decreased in response to inhibition from 48.6 to 47.3 L/min (N.S.).

(3) The computed main effect of exercise on $\dot{V}_{peak}$ was an increase from 32.1 L/min at rest to 48.0 L/min during exercise (p < .01).

(4) There was a significant interaction of resistance loading with exercise on $\dot{V}_{peak}$ (E * R, p < .002); that is, the effect of resistance loading on $\dot{V}_{peak}$ was substantially different at rest compared to resistance loading during exercise; therefore, the effect of resistance on $\dot{V}_{peak}$ should be interpreted separately at rest and during exercise:

At rest, resistance loading decreased $\dot{V}_{peak}$ by 5.1 L/min from 34.6 L/min to 29.5 L/min (15%, p < .05). During exercise resistance loading decreased $\dot{V}_{peak}$ by 13.3 L/min, from 54.6 L/min to 41.3 L/min (25%, p < .05). It is clear from this result that the non-linear nature of

the external resistance load changes the pattern of inspiration much more with the high ventilatory flows produced during exercise (i.e., the resistance is greater during exercise, as shown in the calibration curve, Figure IID).

   f) Peak Inspiratory Mask Pressure (P_{mask}, Figure III 3[1], panel A).

   (1) The computed main effect of inspiratory resistance loading on P_{mask} was a significant pressure drop from -0.64 mmHg to -2.26 mmHg after addition of the inspiratory load (p < .001). This pressure drop and the corresponding peak flow were used to verify the actual in situ resistance calibration for each trial.

   (2) The computed main effect of cholinesterase inhibition on P_{mask} was very small (-1.37 to -1.53 mmHg) but statistically significant owing to the precision of the measurement. The small decrease is a reflection of the slight average increase in peak flow resulting from cholinesterase inhibition.

   (3) The computed main effect of exercise on P_{mask} was a significant increase from -1.08 mmHg at rest to -1.82 mmHg during exercise (p < 0.001); again, the pressure drop is indicative of increased inspiratory flow rates with exercise.

   (4) A significant resistance with exercise (E*R) interaction on P_{mask} was computed (p < .02) and is a result of the increased peak flow with exercise and the non-linear

nature of the 6mm orifice resistor (refer to Figure IID,
Resistance Calibration Curve).

g) Peak Inspiratory Pleural Pressure (PIP$_{PL}$,

Figure III B[1], panel A).

(1) The computed main effect of inspiratory re-
sistance loading on PIP$_{PL}$ was a pressure drop from -14.7 mmHg
to -16.8 mmHg (p < .05). This observation, together with
the increased T_i/T_{tot}, combine to form an increased pleural
pressure-time product (PPTI, an index of the muscular work of
breathing).

(2) The computed main effect of cholinesterase
inhibition on PIP$_{PL}$ was a pressure drop from -13.9 mmHg to
-17.6 mmHg. This difference was statistically significant
(p < .05) and is the major contributor to the increased PPTI,
since T_i/T_{tot} had not significantly changled with this challenge.

(3) The computed main effect of exercise on
PIP$_{PL}$ was a pressure drop from -12.4 mmHg at rest to -19.1 mmHg
during exercise (p < .05).

(4) A significant interaction of resistance with
exercise (E*R) was computed (p < .003), therefore the rest
versus exercise effects of resistance on PIP$_{PL}$ are presented
for contrast: a) at rest, resistance loading produced a
1.0 mmHg drop of PIP$_{PL}$ (N.S.); b) during exercise, however,
the measured pressure drop averaged 3.4 mmHg (p < .05). This
observation is indicative of the exacerbated stress of re-
sistance loading during exercise. It is not unreasonable to

predict this interaction based on the non-linear nature
of our flow resistive load.

h) Peak Inspiratory Pleural Pressure (PEP_{PL})

(1) The computed main effect of inspiratory
resistance loading on PEP_{PL} was negligible (N.S.). This
observation, in the absence of an expiratory flow recording,
is presumptive evidence of unchanged expiratory mechanics
during inspiratory loading.

(2) The computed main effect of cholinesterase
inhibition on PEP_{PL} was an average increase from -2.7 mmHg
before dosing to -1.5 mmHg after (N.S.). This change, though
not significantly large, probably refects the onset of ex-
piratory muscular work resulting from cholinergic airway
constriction in the face of increased mean flow.

(3) The computed main effect of exercise on PEP_{PL}
was a significant increase from -4.0 mmHg at rest of -0.2 mmHg
during exercise (p < .01). This increase in expiratory pressure
is due to the increased ventilatory flows during exercise and
reflects a change from passive to active expiratory mechanisms
(i.e., increased active muscular energy expenditure of the
accessory muscles of breathing).

(4) There were no significant interactions among
the three main treatment variables on peak expiratory pleural
pressure.

i) The Pleural Pressure-Time Index (PPTI; refer to
Figure IIIC[1], panel A).

(1) The computed main effect of inspiratory resistance loading on PPTI was a 21% increase in the muscular work of breathing, compared to the unloaded conditions (p < .05). While there was not a statistically significant interaction between resistance loading and cholinesterase inhibition, it should be noticed that the resistance-induced increase in muscle work was in general relatively less after cholinesterase inhibition than the increase in muscle work observed from resistance alone.

(2) The computed main effect of cholinesterase inhibition on muscular work of breathing (PPTI) was a 46% increase (p < .05), compared to the pre-dose conditions. This increased work reflects mostly increased airways resistance (bronchoconstriction), since ventilation overall was unchanged.

(3) The computed main effect of fixed treadmill exercise on PPTI was a 131% increase (p < .05), most of which is the result of increased ventilation. It also reflects the increased tension required to overcome elastic recoil at the higher lung volumes achieved during exercise inspirations.

(4) There were no statistically significant interactions among the three main treatment variables on PPTI.

j) The Breathing Effectiveness Index (BEI; refer to Figure III C[1], panel B).

(1) The computed main effect of inspiratory resistance loading on breathing effectiveness was a 28% decrement (p < .05), indicating that it took more muscular work to

provide less ventilation when the resistance was in place
in the respirator mask.

(2) The computed main effect of moderate cholin-
esterase inhibition on the BEI was a 32% fall ($p < .05$).
Unlike, the resistance load, this fall in effectiveness of
the respiratory muscles reflects mostly increased airway
resistance secondary to cholinergic bronchoconstriction and
airway glandular secretions, since minute ventilation overall
did not diminish (at rest $\dot{V}_{min}$ increased by 21%).

(3) The computed main effect of exercise on the
BEI was a 34% decrease in the breathing effectiveness index
($p < .05$). This result may appear surprising at first, but
one must appreciate the nature of the forces which must be
overcome when ventilating the lungs at the faster rates and
larger tidal volumes that accompany exercise. Elastic recoil
and tissue viscous forces of both the lung and chest wall
represent a much larger proportion of the "work of inspiring"
than do flow resistance forces. Therefore it is logical
that proportionately more muscle force must be generated, in
overcoming these opposing forces at higher lung volumes, to
promote an incremental increase in ventilation. Since this
index expresses the relationship: unit of ventilation/unit of
expanding pressure, it should not be surprising to this result.

(4) There were no statistically significant inter-
actions among the three main treatment variables on the BEI.

B. Cardiovascular Functions at Rest and Exercise (Table
    IIIB and Figures III D, E, F, G, H).

1. Inspiratory Resistance Loading

    a) Inspiratory resistance loading at rest (RES)
decreased heart rate by 5.8 beats/min (5%) and increased
stroke volume very slightly (0.1 ml), resulting in a re-
duced cardiac output from a control of 2219 ml/min to
2091 ml/min, a reduction of 123 ml/min (6%; refer to Figure
IIID, panels A, B, C).

    Mean aortic blood pressure was unchanged at 100 mmHg,
but mean pulmonary artery pressure fell from a control of
14.9 mmHg to 13.3 mmHg with inspiratory resistance. Mean
left atrial pressure fell from 1.9 mmHg to 1.4 mmHg and mean
right atrial pressure fell from -1.1 mmHg to -1.7 mmHg.

    Mean transmural aortic pressure measured at the
end of diastole (TMP_{ao},diast), which was taken to be the
afterload to left ventricular ejection, remained essentially
unchanged in response to inspiratory loading at rest. Mean
transmural pulmonary artery pressure (TMP_{PA}), which was
taken to be the afterload to right ventricular ejection,
fell from a control value of 21.9 mmHg to 21.0 mmHg. Mean
transmural left atrial pressure (TMP_{LA}), which was taken as
preload or filling pressure for the left ventricle, rose
slightly from 8.7 mmHg to 9.0 mmHg. Mean transmural right
atrial pressure, preload for the right ventricle, also rose

98

TABLE IIIB. CIRCULATORY PARAMETERS, SIMPLE EFFECTS.

PARAMETER	ABBREV.	UNITS	CON	RES	DOS	R*D	EXC	E*R	E*D	E*R*D
			←——— REST ———→				←——— EXERCISE ———→			
Heart Rate	HR	beats/min	110.9±4.7	105.1±4.9	96.0±4.2	94.5±4.4	139.9±6.4	139.3±5.8	122.6±5.0	124.1±4.7
Stroke Volume	SV	ml	20.6±1.3	20.7±1.6	21.9±1.4	22.1±1.6	22.4±1.2	22.5±1.2	22.8±1.2	23.0±1.1
Cardiac Output	$\dot{Q}_{ao}$	ml/min	2219±62.9	2091±65.7	2043±70.8	2023±88.1	3052±97	3058±69.7	2748±102.6	2867±91.2
Aortic Pressure,Syst.	$P_{ao.syst}$	mmHg	134±9.8	131±6.4	134±5.5	130±5.1	150±.	151±7.8	148±7.2	148±7.5
Aortic Pressure,Diast.	$P_{ao.diast}$	mmHg	83±3.5	83±3.4	76±3.3	74±2.8	91±4.5	94±4.4	83±4.1	86±4.4
Mean Aortic Pressure	$\overline{P}_{ao}$	mmHg	100±4.2	99±4.2	95±3.8	93±3.4	111±5.6	113±5.4	105±4.8	107±5.3
Mean Pulmonary Artery Pressure	$\overline{P}_{pa}$	mmHg	14.9±0.6	13.3±0.6	15.1±1.0	13.7±0.9	17.5±1.0	15.7±0.9	16.4±1.1	15.2±1.1
Mean Left Atrial Pressure	$\overline{P}_{la}$	mmHg	1.9±1.2	1.4±1.0	3.9±1.7	2.5±1.6	2.7±1.0	1.9±1.0	4.1±1.9	3.5±1.8
Mean Right Atrial Pressure	$\overline{P}_{ra}$	mmHg	-1.1±0.7	-1.7±0.7	0.1±1.1	-1.3±1.1	-1.3±0.6	-2.4±0.6	-0.5±1.2	-1.7±1.1
Transmural Mean Aortic Pressure, diast.	$\overline{TMP}_{ao.diast}$	mmHg	89.8±5.0	90.0±4.7	83.2±4.9	82.0±4.9	98.2±7.6	102.8±7.1	91.2±5.5	96.0±6.5
Transmural Mean Pulmonary Artery Pressure	$\overline{TMP}_{pa}$	mmHg	21.9±0.9	21.0±1.2	23.7±1.7	22.9±1.6	25.4±2.2	25.6±1.4	25.7±0.8	24.5±1.5
Transmural Mean Left Atrial Pressure	$\overline{TMP}_{la}$	mmHg	8.7±1.1	9.0±1.0	12.6±1.6	11.4±1.7	11.1±1.0	11.7±1.5	13.6±1.8	14.6±2.0
Transmural Mean Right Atrial Pressure	$\overline{TMP}_{ra}$	mmHg	5.9±0.8	6.1±0.7	8.8±1.1	8.0±0.9	7.6±0.8	7.6±0.6	8.7±0.8	9.2±0.9
Left Ventricular Work Rate	$\dot{W}_{lv}$	Kg-M/min	2.53±.11	2.39±.09	2.12±.12	2.11±.13	3.71±.41	4.05±.31	3.17±.29	3.42±.33

FIGURE Ⅲ D

HEART
RATE
(b/min)

A

STROKE
VOLUME
(ml)

B

CARDIAC
OUTPUT
(ml/min)

C
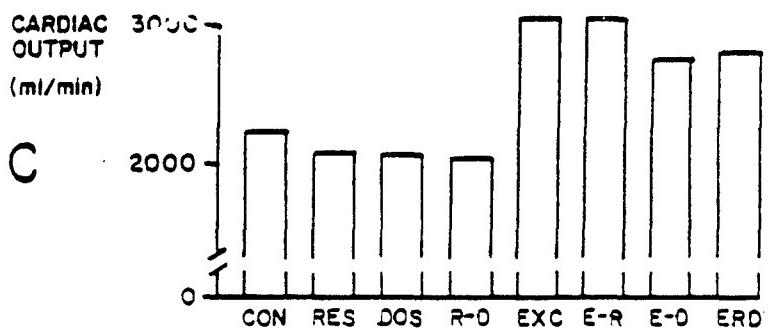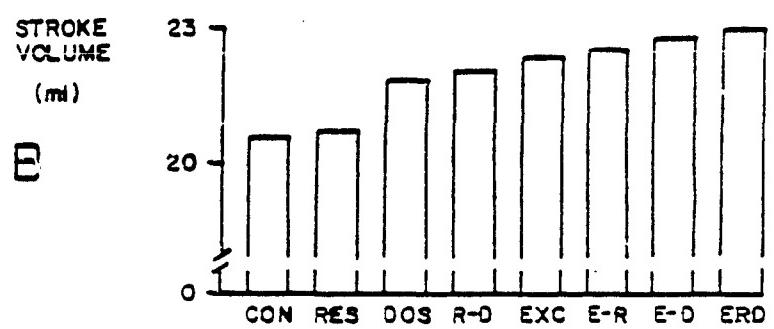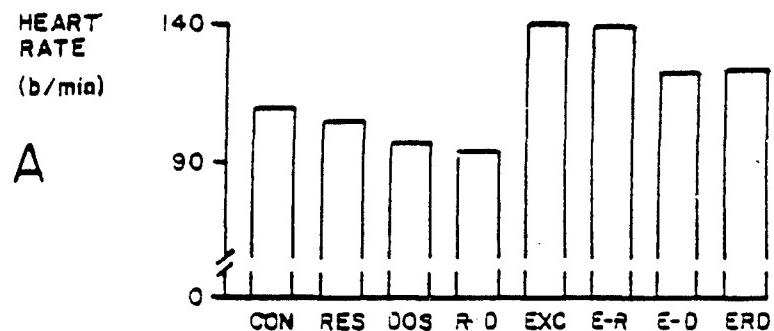
Figure III-D.   Alterations in Cardiac Output Parameters,

Simple Effects.

        Panel A depicts the changes in heart rate associated with

each of the eight challenge conditions. Note the consistently

lower heart rates (relative to the rest and exercise control

levels) associated with cholinesterase inhibition (i.e.,

DOS, R-D and E-D, ERD respectively). Panel B demonstrates

the partial compensation of an increased stroke volume

to the falling HR in the dosed conditions; and panel C

that compensation to be incomplete, since cardiac output

is always lower after dosing than the respective rest and

exercise control values (CON, EXC respectively).

FIGURE III D'

HEART RATE (b/min)

A

STROKE VOLUME (ml)

B

CARDIAC OUTPUT (ml/min)

C
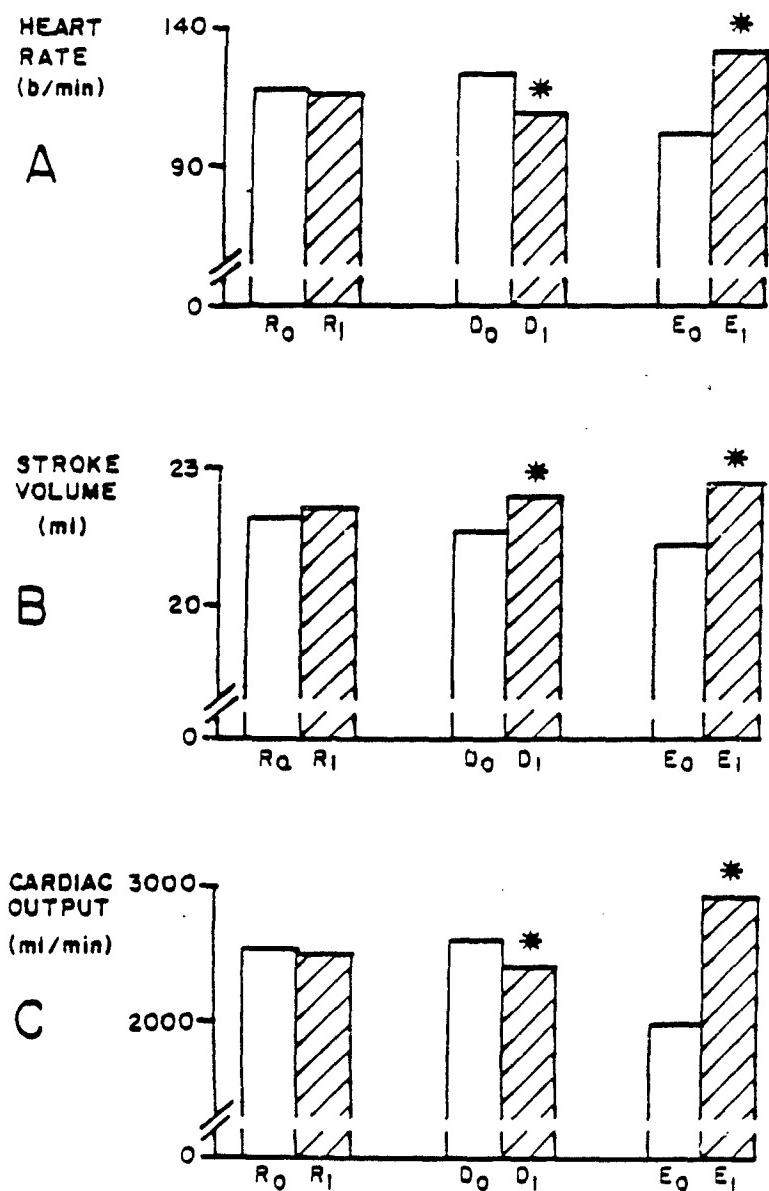
Figure III-D'. Alterations in Cardiac Output Parameters,

Treatment Main Effects.

Panels A, B, C, depict the treatment main effects on
heart rate, stroke volume, and cardiac output, respectively.
A significant interaction was discovered by the analysis of
variance procedure between cholinesterase inhibition and
exercise (D_1 x E_1) on cardiac output. Therefore the main
effects data should not be interpreted directly, but should
be analyzed separately at rest and during exercise for the
cholinesterase inhibition effect. This separate analysis
is presented in Table III-B' and discussed in the text.

FIGURE III E
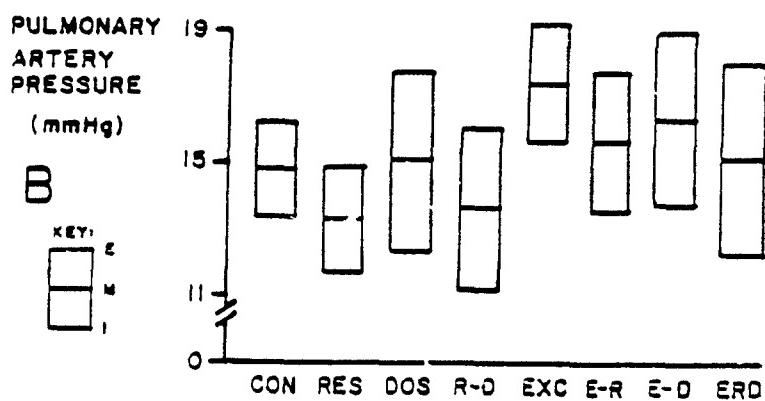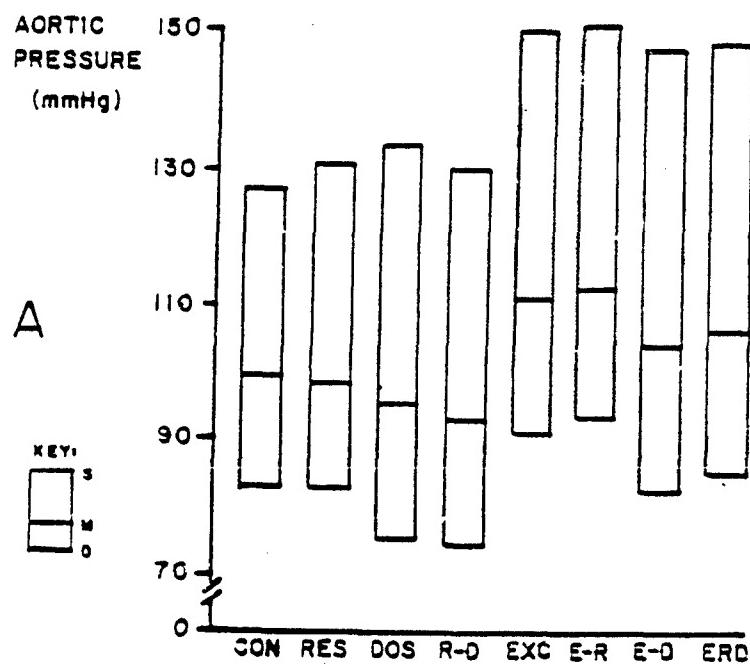
AORTIC PRESSURE (mmHg)

A

KEY: S / M / D

CON RES DOS R-D EXC E-R E-D ERD

PULMONARY ARTERY PRESSURE (mmHg)

B

KEY: E / M / I

CON RES DOS R-D EXC E-R E-D ERD

Figure III-E.   Alterations in Aortic and Pulmonary

Artery Pressures, Simple Effects.

Panel A presents the alterations of aortic blood

pressure (s = systolic, m = mean, d = diastolic;

measured relative to atmospheric pressure) in response

to the eight experimental challenge conditions.

Panel B presents the alterations of pulmonary artery

pressure (e = at peak expiration, m = mean, i = at

peak inspiration; measured relative to atmospheric

pressure) in response to the eight experimental

challenge conditions.

FIGURE III E'
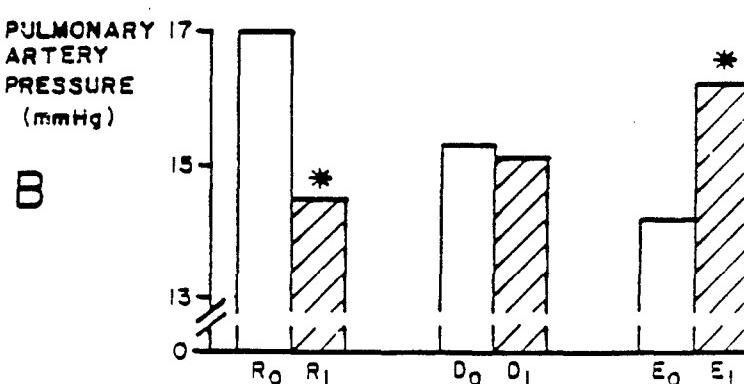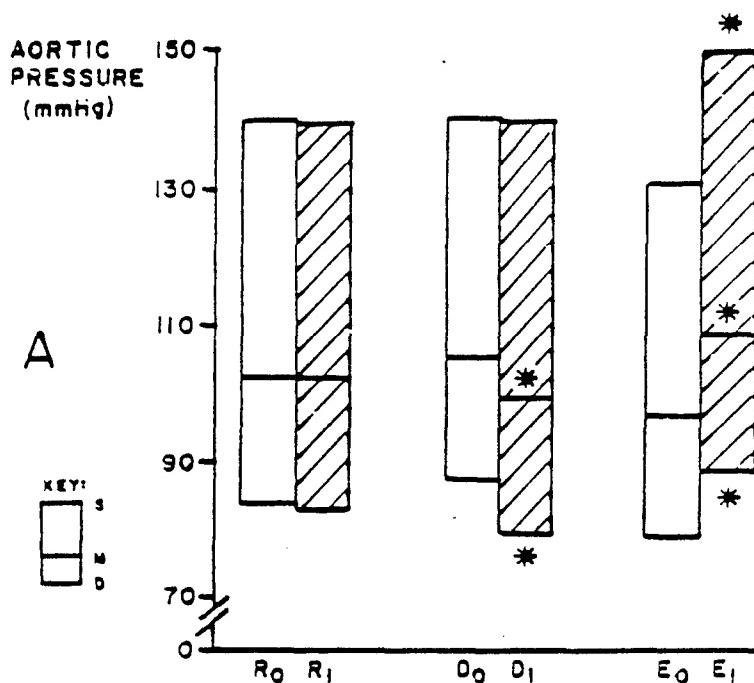
Figure III-E'. Alterations in Aortic and Pulmonary

Artery Pressures, Treatment Main Effects.

Panel A depicts the treatment main effects on systolic,

diastolic, and, mean aortic blood pressure (s, d, and m,

respectively; measured relative to atmospheric pressure).

Panel B depicts the treatment main effects on mean

pulmonary artery pressure, measured relative to atmospheric

pressure.

FIGURE III F
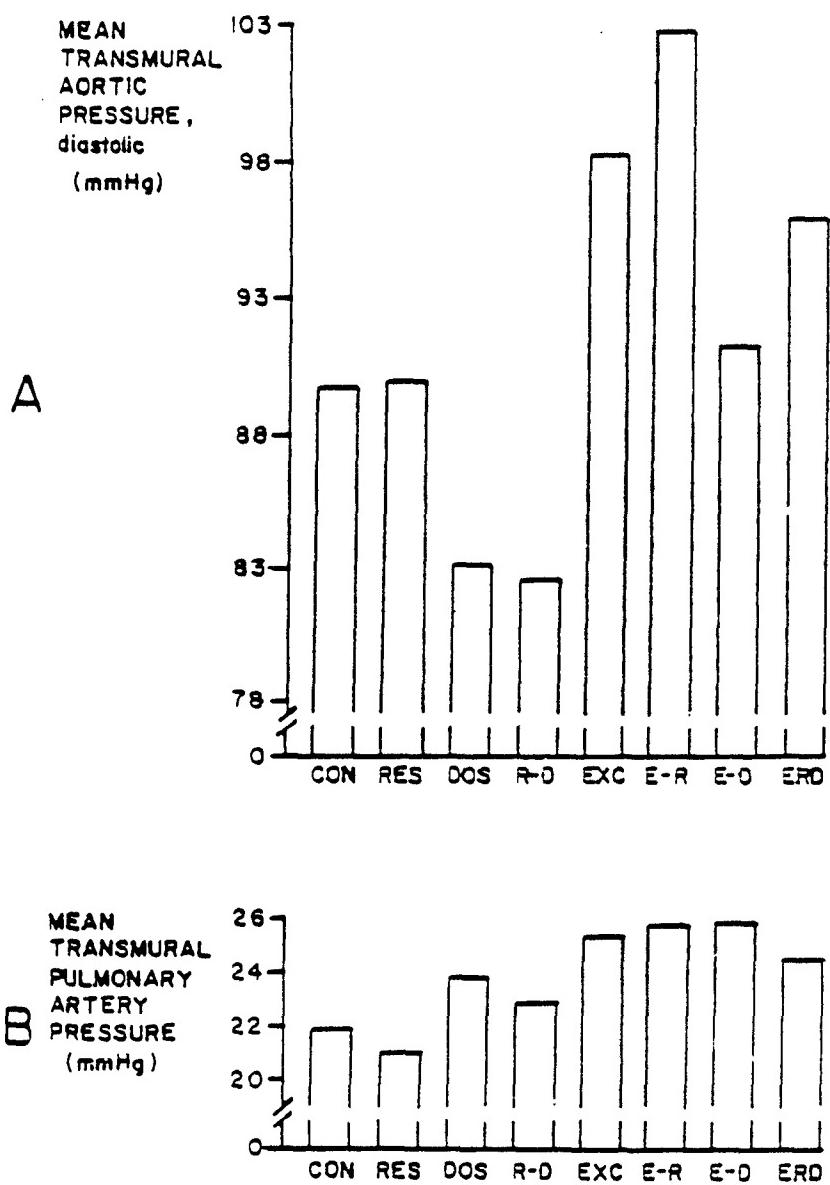
MEAN
TRANSMURAL
AORTIC
PRESSURE,
diastolic
(mmHg)

A

MEAN
TRANSMURAL
PULMONARY
ARTERY
PRESSURE
(mmHg)

B

Figure III-F.   Alterations in Transmural Aortic and
                 Pulmonary Artery Pressures. Simple
                 Effects.

Panel A presents the alterations in diastolic mean
transmural aortic pressure ($\overline{TMP}_{ao}$, diast.; measured
relative to intra-pleural pressure) brought about by
the eight experimental challenge conditions. This
parameter is the effective afterload to left ventricular
ejection. Note the increased afterload associated with
resistance loading, especially during exercise; and
note the decrease in afterload associated with the
cholinesterase inhibited conditions, both at rest
and during exercise. Panel B presents the mean
transmural pulmonary artery pressure responses.

FIGURE III F'

MEAN
TRANSMURAL
AORTIC
PRESSURE,
diastolic
(mmHg)

A

98
93
88
83
78
0

R_O R_I D_O D_I E_O E_I

MEAN
TRANSMURAL
PULMONARY
ARTERY
PRESSURE
(mmHg)

B

26
24
22
20
0

R_O R_I D_O D_I E_O E_I
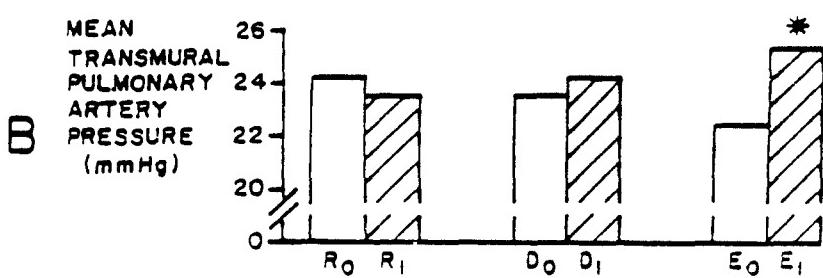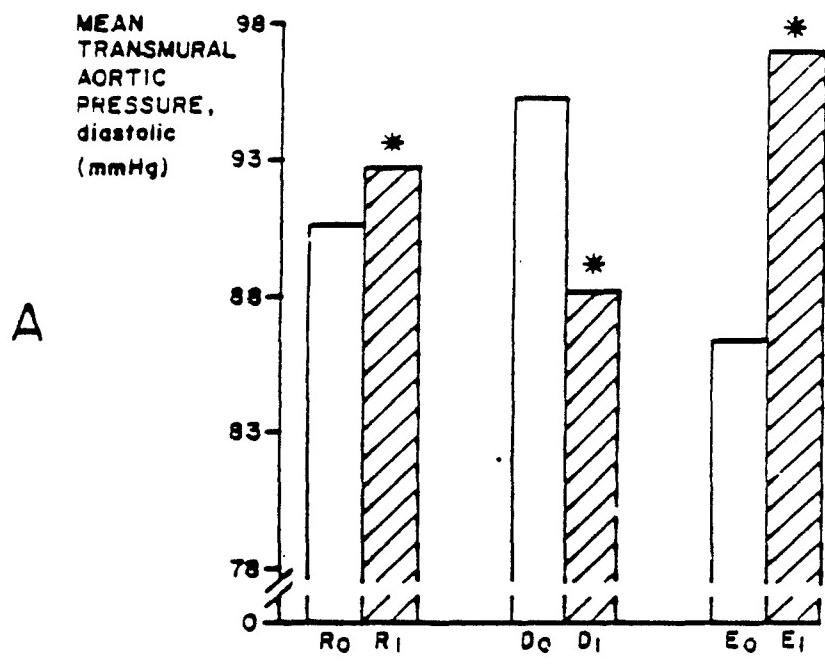
Figure III-F'.   Alterations in Transmural Aortic and

Pulmonary Arterial Pressure,

Treatment Main Effects.

Panel A depicts the significant rise in left heart afterload ($\overline{TMP}_{ao}$, diast.) associated with resistance loading (R_1), the significant fall associated with cholinesterase inhibition (D_1) and the significant rise associated with exercise. The resistance effect, however, was observed during exercise but not at rest and the analysis of variance showed this to be a significant interaction (see Table III-B'). Panel B shows that afterload to the right heart was not similarly altered except by exercise.

FIGURE III G

A — RIGHT ATRIAL PRESSURE (mmHg), with values from 5 to -10 on the y-axis. KEY box labeled E, M, I. Groups on x-axis: CON, RES, DOS, R-D, EXC, E-R, E-D, ERD

B — MEAN TRANSMURAL RIGHT ATRIAL PRESSURE (mmHg), with values 0, 5, 7, 9 on the y-axis. Groups: CON, RES, DOS, R-D, EXC, E-R, E-D, ERD

C — MEAN TRANSMURAL LEFT ATRIAL PRESSURE (mmHg), with values 0, 8, 11, 14 on the y-axis. Groups: CON, RES, DOS, R-D, EXC, E-R, E-D, ERD
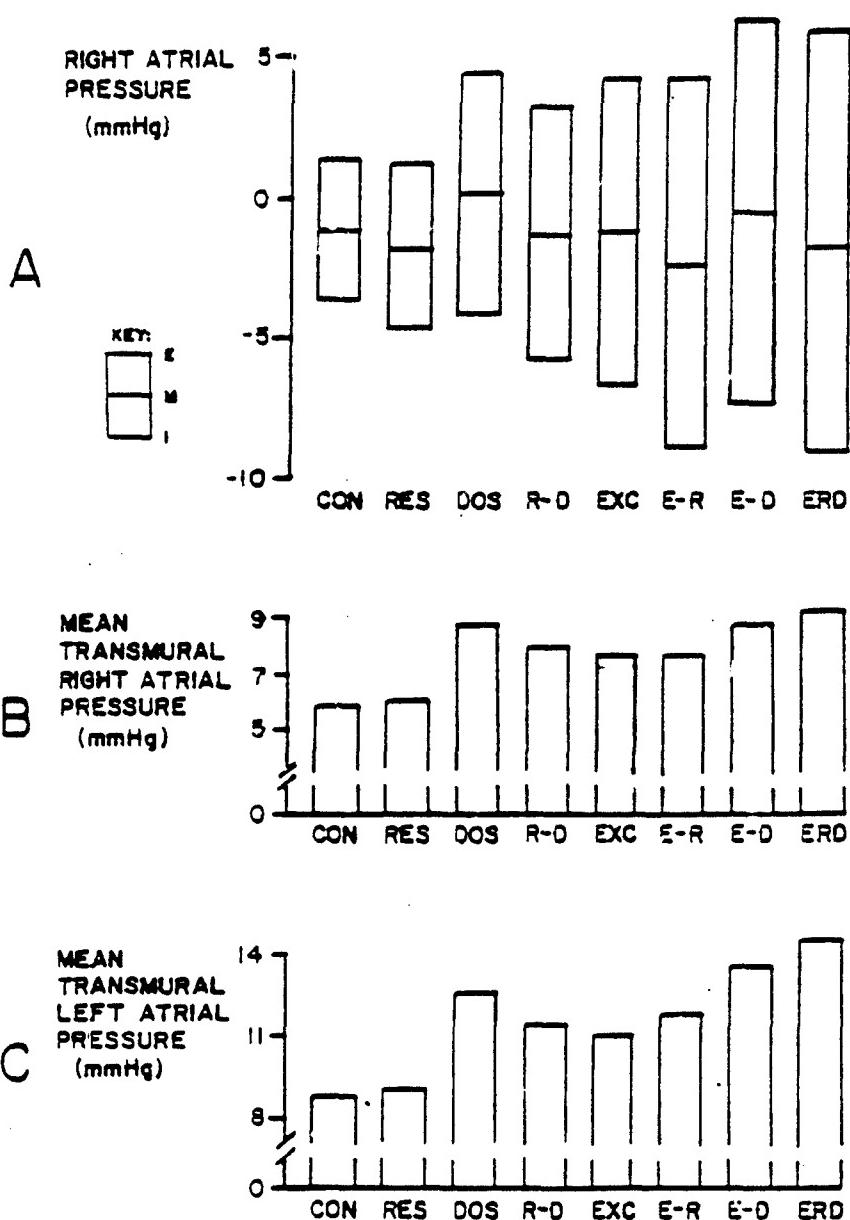
Figure III-G.   Alterations in the Atrial Pressures, Simple

Effects.

Panel A presents right atrial pressure (P_{RA}) at peak

expiration (E) and at peak inspiration (I), as well as the

mean (M) P_{RA} that results, throughout the eight experimental

challenge conditions. This pressure is presented relative

to atmospheric pressure, and as such is the downstream

pressure for venous return. Thus increases in P_{RA}, even

those that occur only during expiration (as during all of the

dosed conditions), can act to impede the venous return

to the heart. Panel B presents mean transmural right atrial

pressure ($\overline{TMP}_{RA}$; right heart preload or filling pressure) as

it changes in response to the eight experimental conditions.

Note the trend of elevated pressures associated with

cholinesterase inhibition (e.g., the DOS, R-D, E-D, and

ERD conditions) compared to their respective rest or exercise

control levels. Panel C presents mean transmural left atrial

pressure ($\overline{TMP}_{LA}$) during the eight condition protocol. Note

the same general trend of elevated left atrial pressures

associated with cholinesterase inhibition that were observed

for the right heart in panel B.

FIGURE III G'

A — MEAN TRANSMURAL LEFT ATRIAL PRESSURE (mmHg), with values at 13, 12, 11, 10, 9, 0 on the y-axis and groups R_0 R_1, D_0 D_1, E_0 E_1 on the x-axis. Asterisks (*) mark D_1 and E_1.

B — MEAN TRANSMURAL RIGHT ATRIAL PRESSURE (mmHg), with values at 8.7, 7.7, 6.7, 0 on the y-axis and groups R_0 R_1, D_0 D_1, E_0 E_1 on the x-axis. Asterisks (*) mark D_1 and E_1.
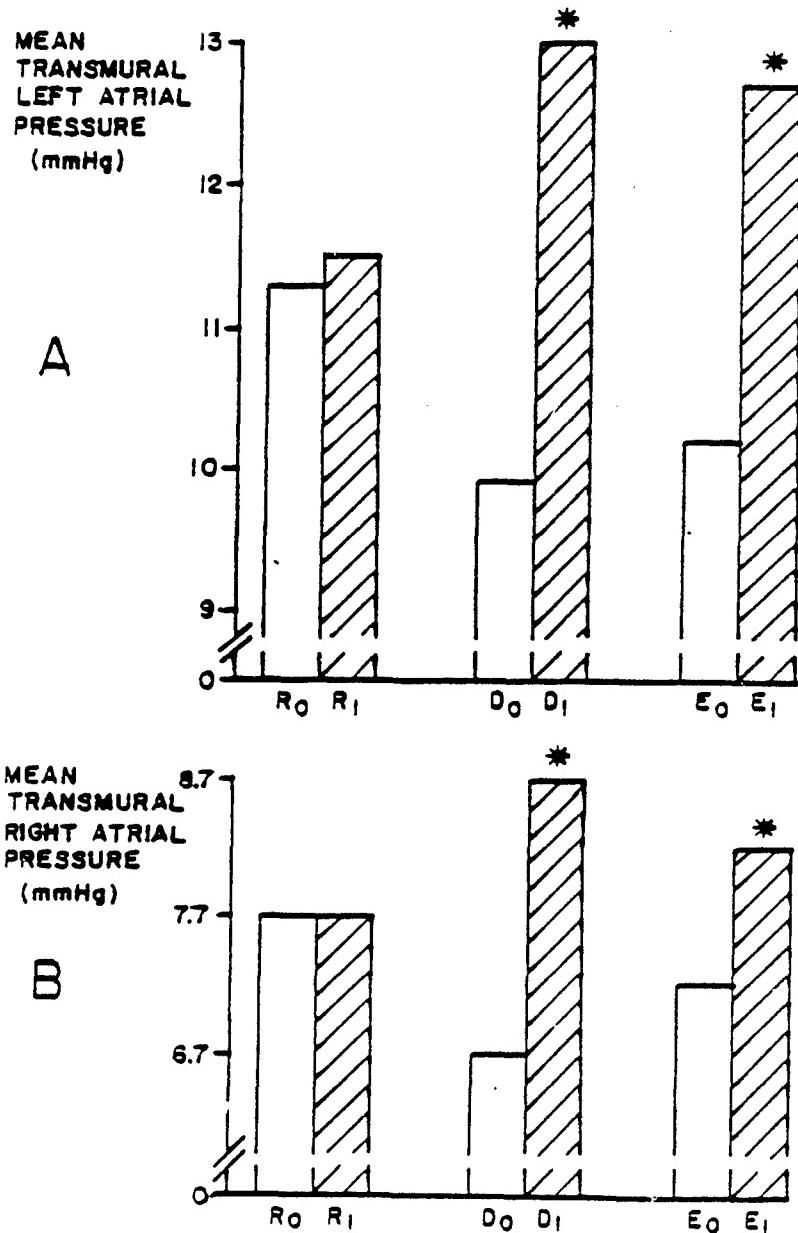
Figure III-G'. Alterations in the Atrial Pressures, Treatment

Main Effects.

Panel A depicts the treatment main effects on $\overline{TMP}_{LA}$

(left heart filling pressure, or preload). The cholinesterase

inhibition effect (D_1 vs D_0) is large, and statistically

significant. The effect of exercise is also significant

but it is associated with the increased cardiac output

brought on by the exercise period. Panel B depicts the

treatment main effects on $\overline{TMP}_{RA}$ (right heart filling pressure,

or preload). The pattern of effects is similar to that in

$\overline{TMP}_{LA}$, but of somewhat smaller magnitude.

FIGURE III H
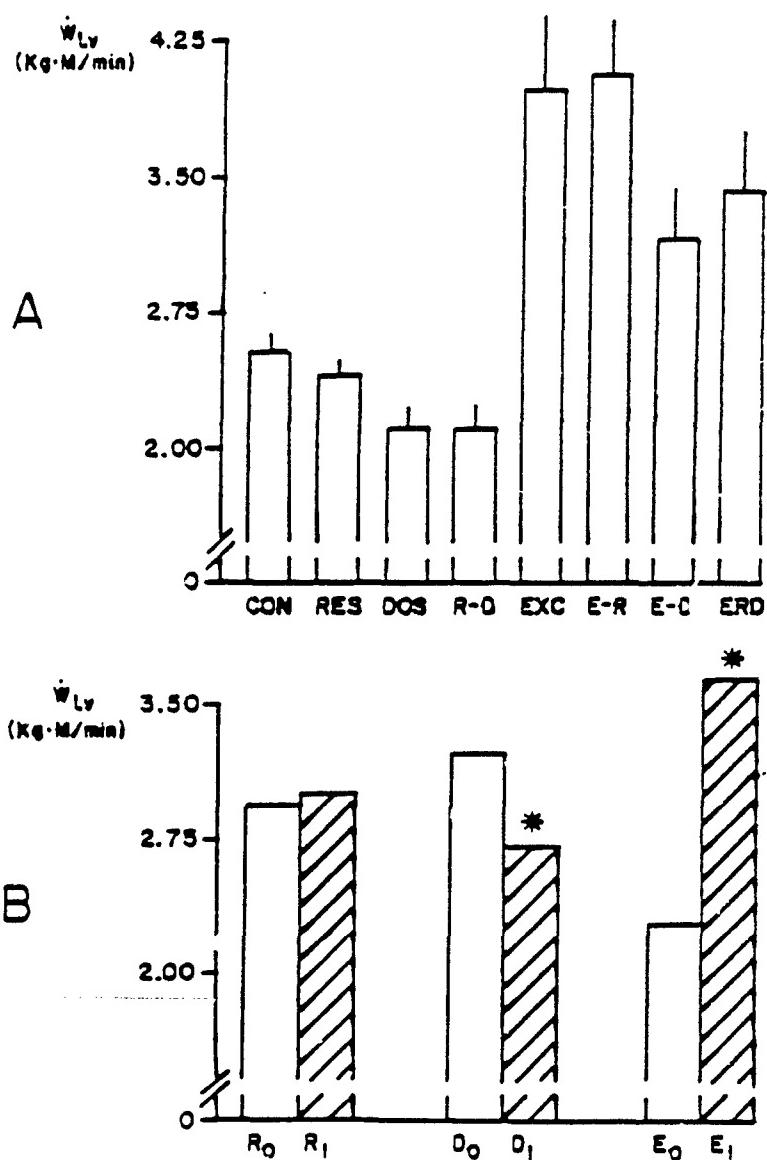
Figure III-H. Alterations in Left Ventricular Work Rate

Panel A presents $\dot{W}_{LV}$ levels associated with each of the eight experimental conditions (i.e., the simple effects). Panel B depicts the treatment main effects on $\dot{W}_{LV}$. It is clear from these two figures that cholinesterase inhibition significantly decreases the work output of the heart, while resistance loading has no consistent significant effect. Note, however, that resistance loading during exercise (E-R and ERD conditions in panel A) consistently increase $\dot{W}_{LV}$ compared to each exercise condition that preceded the addition of an external resistance load. Referring back to Figure III-F, panel A, it is readily apparent that the resistance load conditions shown here during exercise were associated with large increases in afterload to left ventricular ejection ($\overline{TMP}_{ao}$ diast.), thus contributing to the calculated increases in $\dot{W}_{LV}$ from inspiratory resistance loading during exercise.

slightly, from 5.9 mmHg at control to 6.1 mmHg during inspiratory loading. These small increases in ventricular filling pressures may represent reflex decreases in cardiac function, as HR also fell slightly. Left ventricular work rate ($\dot{W}_{LV}$) was not changed.

b) Inspiratory resistance loading during tread-mill exercise (E*R) did not change heart rate and elicited a very small increase in stroke volume (0 1 ml), resulting in a very slight increase in calculated cardiac output from an exercise control of 3052 ml to 3058 ml/min during loading. None of these small changes were significant; rather, the fact that they remain essentially unchanged is the surprising observation, since the additional muscular work requirements of the respiratory muscles (as evidenced by a significantly increased PPTI) would be expected to generate measureable increases in cardiac output.

Mean aortic blood pressure rose slightly from 111 to 113 mmHg, whereas mean pulmonary artery pressure fell from 17.5 to 15.7 mmHg. Mean left atrial pressure fell from 2.7 to 1.9 mmHg, and mean right atrial pressure fell from -1.3 to -2.4 mmHg, largely as a mechanical consequence to the lower mean pleural pressure that results from inspiratory resistance loading.

Mean transmural aortic pressure at end-diastole rose from 98.2 to 102.8 mmHg with the resistance loading during exericse, an afterloading phenomenon which increases

cardiac stroke work and is a possible factor in limiting
maximal work performance while wearing respiratory protective
masks. Mean transmural pulmonary artery was increased only
very slightly (0.2 mmHg). Mean transmural left atrial
pressure rose slightly from 11.1 to 11.7 mmHg, while mean
transmural right atrial pressure remained unchanged at
7.6 mmHg. Left ventricular work rate was increased by
9%, due largely to the resistance-induced increase in
afterload to left ventricular ejection.

2.   Cholinesterase Inhibition.

a) Cholinesterase inhibition (50%) at rest (DOS)
decreased heart rate by 14.9 beats/min (13%) and increased
stroke volume from 20.6 to 21.9 ml. The result was an 8%
decrease in cardiac output from a control of 2219 ml/min to
2053 ml/min during inhibition (refer to Figure III D).

Mean aortic blood pressure fell from 100 to 95 mmHg,
commensurate with the decreased cardiac output, assuming no
change in the tone of the arterial bed. Mean pulmonary
artery pressure was essentially unchanged from the pre-dose
control. Mean left atrial pressure rose from 1.9 to 3.9 mmHg
and mean right atrial pressure rose from -1.1 to +0.1 mmHg,
in spite of the more negative mean pleural pressure.

Mean transmural aortic pressure, diastolic, fell
from a control of 89.8 mmHg to 83.2 mmHg after administration
of neostigmine (DOS). This diminished afterload in the
presence of an increased preload to the left ventricle

(mean transmural left atrial pressure rose from 3.7 to 12.6 mmHg), is evidence of diminished myocardial performance secondary to the neostigmine-induced vagal tone. On the other hand, mean transmural pulmonary artery pressure rose from 21.9 to 23.7 mmHg, while mean transmural right atrial pressure rose from 5.9 to 8.8 mmHg. Again, the increased preload or filling pressure to the right side of the heart is indicative of a weakened contractile ability of the right ventricular myocardium, whereas the increase in pulmonary artery pressure relative to pleural pressure (TMP_{PA}) is probably a result of pooling or congestion of blood in the lungs caused by the cholinergically weakened heart. If this interpretation is correct, the resulting increased afterload to right ventricular ejection must certainly contribute to right heart stroke work requirements at a time when it is least capable of compensating. Left ventricular work rate, $\dot{W}_{LV}$, was diminished by 16%, demonstrating that the heart was doing less work under these conditions than it was prior to the inhibition.

b) Cholinesterase inhibition during treadmill exercise (E*D) reduced heart rate from 139.9 to 122.6 beats/min (12%) and stroke volume increased from 22.4 to 22.8 ml in partial compensation, resulting in a 10% decrease of cardiac output (from predose exercise level of 3052 ml/min to 2748 ml/min).

Mean aortic blood pressure fell from 111 to 105 mmHg and mean pulmonary artery pressure decreased from 17.5 to 16.4 mmHg.

Mean left atrial pressure rose from 2.7 to 4.1 mmHg and
mean right atrial pressure became less negative from
-1.3 to -0.5 mmHg. Again, the increased arterial pressures
in the presence of a more negative intrathoracic pressure
is indicative of a changed myocardial function.

Mean transmural aortic pressure, diastolic, dropped
from 98.2 to 91.2 mmHg and mean transmural pulmonary artery
pressure rose slightly (0.3 mmHg). This pattern is similar
to that which occurred at rest during cholinesterase in-
hibition.

Mean transmural left atrial pressure rose from
11.1 to 13.6 mmHg and mean transmural right atrial pressure
rose from 7.6 to 8.7 mmHg. Again, this pattern of changed
myocardial function was basically similar to that which
occurred at rest except that the increases of left and right
preload were relatively smaller during exercise. Left
ventricular work rate ($\dot{W}_{LV}$) was decreased by 20%.

3. Inspiratory Resistance Loading Combined with
   Cholinesterase Inhibition

a) The combined challenge of inspiratory re-
sistance loading while under cholinesterase inhibition,
at rest (R*D), resulted in a decreased heart rate from
110.9 to 94.5 beats/min, and a partially compensating in-
crease in stroke volume (20.6 to 22.1 ml), resulting in a
10% decrease of cardiac output from 2219 to 2023 ml/min
compared to control (refer to Figure III-D).

Mean aortic blood pressure fell from 100 to 93 mmHg and mean pulmonary artery pressure was reduced from 14.9 to 13.7 mmHg. Mean left atrial pressure rose from 1.9 to 2.5 mmHg and mean right atrial pressure fell very slightly (0.2 mmHg).

Mean transmural aortic pressure, diastolic, fell from 89.8 to 82.0 mmHg, while mean transmural pulmonary artery pressure rose from 21.9 to 22.9 mmHg. Mean transmural left atrial pressure was elevated from 8.7 to 11.4 mmHg, and mean transmural right atrial pressure rose from 5.9 to 8.0 mmHg. It should be noted here that the transmural atrial pressures appear to respond to the combined challenge of inspiratory resistance loading and moderate cholinesterase inhibition by settling at levels that are intermediate between the response levels of either challenge alone. That is, they are not additive in their response as might have been anticipated, but rather represent a compromise of the effects of the two treatment conditions. Left ventricular work rate was identical to that observed with cholinesterase inhibition alone (decreased 16% from control).

b) Resistance loading in combination with moderate cholinesterase inhibition during exercise (E*R*D) slowed heart rate from an exercise control (EXC) rate of 139.9 to an after challenge rate of 124.1 beats/min. Stroke volume was slightly elevated (22.4 to 23.0 ml) and the cardiac output was decreased from 3052 ml/min to 2807 ml/min (8%).

Mean aortic blood pressure was somewhat lower at 107 mmHg compared to 111 mmHg measured prior to challenge. Mean pulmonary artery pressure dropped from 17.5 to 15.2 mmHg. Mean left atrial pressure rose from 2.7 to 3.5 mmHg and mean right atrial pressure was observed to fall from -1.3 to -1.7 mmHg. These observed atrial pressure reflect the summated effects of resistance loading and cholinesterase inhibition acting separately. That is, the more negative pleural pressure of resistance loading tending to pull the atrial pressures down, and the congestive, vagally influenced heart ending to increase these same pressures by inadequate ejection of the ventricles.

Mean transmural aortic pressure, diastolic, dropped by 2 mmHg and mean transmural pulmonary artery pressure fell by approximately 1 mmHg. Transmural mean left atrial pressure rose from 11.1 to 14.6 mmHg and mean transmural right atrial pressure rose from 7.6 to 9.2 mmHg, reflecting the combination of a more negative surrounding pressure (P_{PL}) induced by inspiratory loading, and increased back pressure from the cholinergically weakened ventricles. $\dot{W}_{LV}$ was less than the exercise control by 8%, but was greater than cholinesterase inhibition alone.

4. Main Effects and Interactions of Treatment Variables on Cardiovascular Functions (refer to Table III-3[1] and Figures III-D[1], E[1], F[1], G[1], and H[1]).

a) Heart Rate (HR, refer to Figure III-D[1], panel A).

TABLE IIIB. CIRCULATORY PARAMETERS, MAIN TREATMENT EFFECTS

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PARAMETER	ABBREV.	UNITS	Resistance(R)		ChE Inhibition(D)		Exercise(E)		Interactions (see footnotes)
			R_0	R_1	D_0	D_1	E_0	E_1	
Heart Rate	HR	beats/min	117.4 NS 115.8		123.8 * 109.3		101.6 * 131.5		None
Stroke Volume	SV	ml	21.9 NS 22.1		21.6 * 22.4		21.3 * 22.7		None
Cardiac Output	$\dot{Q}_{ao}$	ml/min	2515 NS 2495		2605 * 2405		2094 * 2916		E x D[1]
Aortic Pressure,Syst.	$P_{ao,syst}$	mmHg	139.9 NS 139.6		139.9 NS 139.6		130.4 * 149.2		None
Aortic Pressure,Diast.	$P_{ao,diast}$	mmHg	84.1 NS 83.1		87.7 * 79.6		78.9 * 88.3		None
Mean Aortic Pressure	P_{ao}	mmHg	102.6 NS 102.5		105.5 * 99.7		96.5 * 108.6		None
Mean Pulmonary Artery Pressure	P_{pa}	mmHg	17.0 * 14.5		15.3 NS 15.1		14.2 * 16.2		None
Mean Left Atrial Pressure	P_{la}	mmHg	3.1 NS 2.3		1.9 * 3.5		2.3 NS 3.0		None
Mean Right Atrial Pressure	P_{ra}	mmHg	-0.7 * -1.8		-1.6 NS -0.8		-1.0 NS -1.5		None
Transmural Mean Aortic Pressure, diast.	$THP_{ao,diast}$	mmHg	90.6 * 92.7		95.2 * 88.1		86.4 * 96.9		E x M[2]
Transmural Mean Pulm. Artery Pressure	THP_{pa}	mmHg	24.2 NS 23.5		23.5 NS 24.2		22.4 * 25.3		None
Transmural Mean Left Atrial Pressure	THP_{la}	mmHg	11.3 NS 11.5		9.9 * 13.0		10.2 * 12.7		None
Transmural Mean Right Atrial Pressure	THP_{ra}	mmHg	7.7 NS 7.7		6.7 * 8.7		7.2 * 8.2		None
Left Ventricular Work	$\dot{W}_{lv}$	Kg·M/min	2.88 NS 2.99		3.23 * 2.70		2.28 * 3.65		None

(1) Cardiac Output D_0 D_1
At test(E_0): 2135*2011
At Exer.(E_1): 3055*2711; i.e., the ChE inhibition effect on $\dot{Q}_{ao}$ was significantly larger during exercise than at rest.

(2) $THP_{ao,diast}$. M_0 M_1
At test(E_0): 80.5*86.3
At Exer.(E_1):94.7*99.1;i.e.,no effect of resistance on THP_{ao} at rest, but during exercise afterload rose 4.4 mmHg.

(1) The computed main effect of inspiratory
resistance loading on HR was a decrease from 117.4 to
115.8 beats/min (N.S.).

(2) The computed main effect of moderate
cholinesterase inhibition on HR was a significant decrease
from 123.8 to 109.3 beats/min (p < .01).

(3) The computed main effect of fixed treadmill
exercise on HR was a significant increase from 101.6 to
131.5 beats/min (p < .01).

(4) There were no statistically significant in-
teractions among the three treatment variables; however,
there was a consistent, albeit small, reduction in HR in
the presence of resistance loading at rest, 5.8 beats/min,
that does not re-occur during exercise (the heart rate change
that would have been required for statistical significance
at the p < .05 level by Duncan's Multiple Range test was
6.8 beats/min). This "borderline" but consistent decrease
in HR at rest is suggestive of a reflex mechanism that has
been observed to reduce heart rate during spontaneous deep
inspirations and during Mueller maneuvers (Schrijen et al,
1975; Fitzgerald et al, 1981).

b) Stroke Volume (SV, refer to Figure III-D[1], panel B).

(1) The computed main effect of inspiratory resis-
tance loading on SV was a slight increase from 21.9 ml to
22.1 ml (N.S.). This response, though too small to be
statistically significant, corroborates the reality of the

finding of decreased HR with resistive loading especially in light of the finding of a small increase in left and right ventricular filling pressures under the same conditions (para. B1, a).

(2) The computed main effect of moderate cholinesterase inhibition on SV was a somewhat larger and statistically significant increase from 21.6 ml to 22.4 ml (p < .05).

(3) The computed main effect of treadmill exercise on SV was a significant increase from 21.3 to 22.7 ml (p < .05).

(4) There were no statistically significant interactions among the three treatment variables with SV.

c) Cardiac Output (Q_{ao}, refer to Figure III-D[1], panel C).

(1) The computed main effect of inspiratory resistance loading on Q_{ao} was a very slight overall decrease from 2515 to 2495 ml/min (N.S.).

(2) The computed main effect of moderate cholinesterase inhibition on Q_{ao} was a significant decrease from 2605 to 2405 ml/min (p < .01).

(3) The computed main effect of treadmill exercise on Q_{ao} was a significant increase in output from 2094 to 2916 ml/min (p < .01).

(4) There was a significant interaction between cholinesterase inhibition and exercise on cardiac output (p < .03). Consequently, the main effect of each should not be interpreted, but rather the simple effect of cholinesterase inhibition at rest is here contrasted with the simple effect

of cholinesterase inhibition during exercise: At rest,
cardiac output was decreased from 2155 ml/min to 2033
ml/min (< 6%) after inhibition (p < .05). During treadmill
exercise, the effect of cholinesterase inhibition on
cardiac output was a significant larger decrease (> 9%)
from 3055ml/min to 2777 ml/min (p <.05). In view of
estimated three to five-fold increases in work of breathing,
under inhibition conditions, this diminished output un-
questionably requires other physiological adjustments
to compensate for the "deficit".

There were no other significant interactions among
the treatment variables with cardiac output.

d) Mean Aortic Blood Pressure (P_{ao}. refer to

Figure III-E[1], panel A).

(1) The computed main effect of inspiratory re-
sistance loading on P_{ao} was negligible (N.S.).

(2) The computed main effect of moderate cholin-
esterase inhibition on P_{ao} was a significant decrease from
105.5 mmHg to 99.7 mmHg (p <.05). This fall in mean aortic
pre sure is commensurate with the overall decrease in
cardiac output under the same conditions and assuming no
cholinergic changes in vascular tone.

(3) The computed main effect of treadmill exercise
on P_{ao} was a significant increase from 96.5 mmHg at rest to
10f 6 during exercise (p <.01).

(4) There were no statistically significant

interactions among the three treatment variables with P_{ao}.

e) Mean Pulmonary Artery Pressure (P_{PA}, refer
to Figure III-E[1], panel 3).

(1) The computed main effect of inspiratory re-
sistance loading on P_{PA} was a significant reduction from
17.0 mmHg without resistance loading to 14.5 mmHg with loading
(p <.05). This reduction reflects the decreased mean pleural
pressure that results from the inspiratory resistance load.

(2) The computed main effect of moderate cholin-
esterase inhibition on P_{PA} was negligible (N.S.).

(3) The computed main effect of treadmill exercise
on P_{PA} was a significant increase from 14.2 mmHg at rest to
16.2 mmHg during exercise (p <.05). Increased pulmonary artery
blood flow and superimposed changes in mean pleural pressure
with exercise as well as increased sympathetic vascular tone
combine to produce this increase.

(4) There was no statistically significant
interaction among the three treatment variables on P_{PA}.

f) Mean Left Atrial Pressure (P_{LA})

(1) The computed main effect of inspiratory re-
sistance loading on P_{LA} was a reduction from 3.1 mmHg before
loading to 2.3 mmHg with the inspiratory resistance added (N.S.).
This small change is undoubtedly the effect of the resistance-
induced decrease in mean pleural pressure.

(2) The computed main effect of moderate cholin-
esterase inhibition on P_{LA} was a significant rise in pressure

from 1.9 mmHg to 3.5 mmHg (p .05). When this pressure

change is measured relative to changing pleural pressure

(TMP_{la}), it represents a large change in left ventricular

preload (see parag. j, this section).

(3) The computed main effect of treadmill

exercise on P_{LA} was an increase from 2.3 mmHg at rest

to 3.0 mmHg during exercise (N.S.).

(4) There was no statistically significant in-

teractions among the three treatment variables on P_{LA}.

g) Mean Right Atrial Pressure (P_{RA}).

(1) The computed main effect of inspiratory re-

sistance loading P_{RA} was a significant decrease from -0.7

to -1.8 mmHg (p .05). Again, since this pressure was re-

corded relative to atmospheric pressure, the changes seen are

largely due to reflected changes in mean pleural pressure in

response to the inspiratory load. This decrease in P_{RA} should

have increased the gradient for venous return and thereby in-

crease the cardiac output (Guyton, 1956). This response was

not observed at rest but the small cardiac output increase

during resistance loading with exercise may be accounted for

by this mechanism.

(2) The computed main effect of moderate cholin-

esterase inhibition on P_{RA} was an increase from -1.6 to

-0.8 mmHg (N.S.). P_{RA} is often used in conjunction with mean

systemic pressure ($P_{ms}-P_{RA}$ = gradient) to establish the gradient

for venous return. Assuming that P_{ms} has not risen (if anything,

it should fall with the decreased Q_{ao} observed after cholinesterase inhibition) this rise in P_{RA} would have decreased the gradient for venous return, adjusting it to the new level of cardiac output induced by neostigmine.

(3) The computed main effect of treadmill exercise on P_{RA} was a decrease from -1.0 mmHg at rest to -1.5 mmHg during exercise (N.S.).

(4) There were no statistically significant interactions among the three treatment variables on P_{RA}.

h) Transmural Mean Aortic Pressure, Diastolic

(TMP_{ao},diast., refer to Figure III-F[1], panel A).

(1) The computed main effect of inspiratory resistance loading on TMP_{ao}diast. was a significant increase in afterload from 90.6 to 92.7 mmHg (p <.05). With cardiac output held essentially unchanged under the same conditions it is apparent that cardiac work rate must have increased a relative amount (filling pressure, TMP_{LA}, was essentially unchanged as well).

(2) The computed main effect of moderate cholinesterase inhibition on TMP_{ao},diast. was a significant decrease in afterload from 95.2 mmHg to 88.1 mmHg (p <.05). This response, as well as the increase in filling pressure, TMP_{la}, in the face of decreased cardiac output under these conditions are further evidence of significantly impaired cardiac (left ventricular) function.

(3) The computed main effect of treadmill exercise on TMP_{ao},diast. was a significant increase in afterload from 86.4 mmHg at rest to 96.9 mmHg during the exercise conditions.

(4) There was a significant interaction of resistance loading with exercise on TMP_{ao},diast. ($p < .03$). Therefore the computed main effect of resistance must be evaluated separately at rest and during exercise:

At rest, the resistance load produced an insignificant and very small decrease in afterload (following the small decrease in cardiac output under the same conditions). During treadmill exercise, the afterload induced by resistance loading rose from 94.7 mmHg unloaded to 99.1 mmHg after the resistance was introduced ($p < .05$). Apparently, the increased respiratory frequency and prolonged inspiratory time of the exercise conditions as well as the increased magnitude of negative inspiratory pleural pressure had summated to produce the markedly increased afterload during exercise that was not apparent under rest conditions.

i) Transmural Mean Pulmonary Artery Pressures

(TMP_{PA}, refer to Figure III-F[1], panel B).

(1) The computed main effect of inspiratory resisance loading on TMP_{PA} was a small decrease from 24.2 to 23.5 mmHg (N.S.).

(2) The computed main effect of moderate cholinesterase inhibition on TMP_{PA} was a small increase from 23.5 to

24.2 mmHg (N.S.) even though the aortic blood pressures had fallen commensurate with the diminished cardiac output. This finding is evidence of increased blood volume in the lungs and left heart.

(3) The computed main effect of treadmill exercise on TMP_{PA} was a significant increase from 22.4 to 25.3 mmHg (p <.05).

(4) There were no statistically significant interactions among the three treatment variables on TMP_{PA}.

j) Transmural Mean Left Atrial Pressure (TMP_{LA}, refer to Figure III-G[1], panel A).

(1) The computed main effect of inspiratory resistance loading on TMP_{LA} was a very slight increase from 11.3 to 11.5 mmHg (N.S.).

(2) The computed main effect of moderate cholinesterase inhibition on TMP_{LA} was a significant increase in left ventricular filling pressure from 9.9 mmHg to 13.0 mmHg after inhibition (p <.05). This increased preload in the presence of a diminished afterload and cardiac output is clear evidence of a severely compromised left ventricular function.

(3) The computed main effect of treadmill exercise on TMP_{LA} was a significant increase in left ventricular filling pressure from 10.2 to 12.7 mmHg (p <.05).

(4) There were no statistically significant interactions among the three treatment variables on TMP_{LA}.

k) Mean Transmural Right Atrial Pressure (TMP_{RA}, refer to Figure III-G', panel 3).

(1) The computed main effect of inspiratory resistance loading on TMP_{RA} was negligible, it remained unchanged at 7.7 mmHg averaged across rest and exercise.

(2) The computed main effect of rate cholinesterase inhibition on TMP_{RA} was a significant increase in right ventricular filling pressure from 6.7 mmHg in the uninhibited conditions to 8.7 mmHg after administration of neostigmine ($p < .05$).

(3) The computed main effect of treadmill exercise on TMP_{RA} was a small but significant increase from 7.2 mmHg at rest to 8.2 mmHg during exercise ($p < .05$). This observation minus the left heart mechanics in that preload is increased while afterload is unchanged, suggesting impaired right ventricular function.

(4) There were no statistically significant interactions among the three treatment variables on TMP_{RA}.

l) Left Ventricular Work Rate ($\dot{W}_{LV}$, refer to Figure III-H, panel 3).

(1) The computed main effect of inspiratory resistance loading on $\dot{W}_{LV}$ was a small increase due primarily to the increase in afterload associated with the more negative pleural pressures that accompanied inspiratory loading especially during exercise. This effect was not statistically significant, but is of some interest in extrapolating this data to the worker

who may have undiagnosed cardiovascular impairment. The increase in $\dot{W}_{LV}$ occurred exclusively during exercise but was of insufficient magnitude to reach the significance criterion.

(2) The computed main effect of moderate cholin-esterase inhibition on $\dot{W}_{LV}$ was a sharp reduction from 3.23 to 2.70 kg·M/min (16%, $p < .05$). This finding has interesting interpretative possibilities. On the one hand, it undoubtedly reflects diminished cardiac function in the face of increased tissue demands (as will be demonstrated by the blood gas data to follow). On the other hand, it also demonstrates that the "stress" or metabolic require-ments of the heart are less. The increased filling pressures ($\overline{TMP}_{LA}$, $\overline{TMP}_{RA}$) during this inhibition also have undoubtedly increased end-diastolic ventricular volume, and thus the myocardium is on a more effective position of the "length-tension curve" of the heart (Guyton, 1955).

(3) The computed main effect of fixed treadmill exercise on $\dot{W}_{LV}$ was a marked increase from 2.28 kg·M/min averaged at rest, to 3.65 kg·M/min during the exercise conditions (60% increase, $p < .05$).

(4) There were no statistically significant in-teractions among the three treatment variables on $\dot{W}_{LV}$, however as stated previously a consistent increase in $\dot{W}_{LV}$ was observed during exercise with resistance loading (approximately 10% during exercise; no change at rest).

C. Blood Gas Parameters at Rest and Exercise

(Table III-C and Figures III-J, K, L, J[1], and K[1])

The simple effects of the eight treatment conditions (2^3 treatment variables) on measured blood gas parameters are presented in Table III-C. Most of the individual changes of these parameters in response to treatment challenges are small; and due to relatively larger inter-animal differences it is difficult to achieve statistical significance. Consequently, summary data will be presented here as main treatment effects on the various blood gas parameters measured, and interactions clarified where pertinent (Table III-C[1]).

1. Arterial Oxygen Tension (P_aO_2) (refer to Figures J and J[1], panel A).

(a) The computed main effect of inspiratory resistance loading on P_aO_2 was negligible (78.6 mmHg before loading, 78.3 mmHg after; N.S.).

(b) The computed main effect of moderate cholinesterase inhibition on P_aO_2 was a significant fall in tension from 81.5 mmHg in the uninhibited conditions to 75.4 mmHg after neostigmine was administered (p <.05). In light of an absence of significant change in overall ventilation under these conditions, the data suggests that there may be localized areas of hypoventilation or shunt.

(c) The computed main effect of treadmill exercise on P_aO_2 was negligible.

TABLE III C. BLOOD GAS PARAMETERS

PARAMETER	ABBREV	UNITS	TREATMENT CONDITION							
			COM	RES	DOS	B+O	EXC	E+R	E+O	E+R+O
Oxygen Tension, art.	P_aO_2	mmHg	81.2±1.2	82.2±1.0	76.4±3.0	74.8±2.9	81.2±2.0	81.2±2.4	75.4±3.3	74.8±3.1
Oxygen Tension, ven.	P_vO_2	mmHg	40.0±1.4	39.4±1.4	37.8±2.0	37.0±1.8	34.6±1.6	35.2±1.7	32.6±1.4	32.0±1.4
Oxygen Content, art.	O_2Ct_a	Vol%	16.3±1.1	16.4±1.1	16.0±1.1	16.0±1.2	16.4±1.0	16.4±1.1	16.0±1.2	16.0±1.2
Oxygen Content, ven.	O_2Ct_v	Vol%	11.4±1.1	11.3±1.1	10.4±1.2	10.2±1.3	9.5±0.9	9.1±1.0	8.7±1.0	8.5±1.0
A-V Difference	$(a-v)O_2Ct$	Vol%	4.9±0.2	5.1±0.4	5.6±0.4	5.8±0.5	6.9±0.2	6.8±0.4	7.3±0.5	7.5±0.6
Oxygen Consumption	$\dot{V}O_2$	ml/min	104±6.4	96±8.3	104±3.1	109±16.0	195±12.3	192±8.1	178±10.3	180±10.6
CO_2 Tension, art.	P_aCO_2	mmHg	37.3±1.2	37.0±1.2	36.7±1.6	37.8±1.4	36.7±1.1	36.1±0.6	37.5±2.1	38.2±1.7
CO_2 Tension, ven.	P_vCO_2	mmHg	41.2±1.5	42.2±1.5	42.3±2.1	43.0±2.0	42.9±1.2	44.0±1.2	44.5±2.4	46.1±1.9
pH, art	pH_a		7.447±.0006	7.443±.008	7.440±.015	7.436±.012	7.452±.007	7.453±.007	7.440±.017	7.433±.015
pH, ven	pH_v		7.428±.010	7.422±.009	7.414±.016	7.410±.013	7.420±.009	7.415±.009	7.410±.015	7.398±.016
Shunt fraction	I_s	%	5.8	3.6	9.7	9.4	2.8	2.7	7.6	7.4

REST ——————— | EXERCISE ———————

FIGURE III J

PO₂ (mmHg)

A

CON RES DOS R-D EXC E-R E-D ERD

PCO₂ (mmHg)

B

CON RES DOS R-D EXC E-R E-D ERD

pH

C

KEY:
A V

CON RES DOS R-D EXC E-R E-D ERD
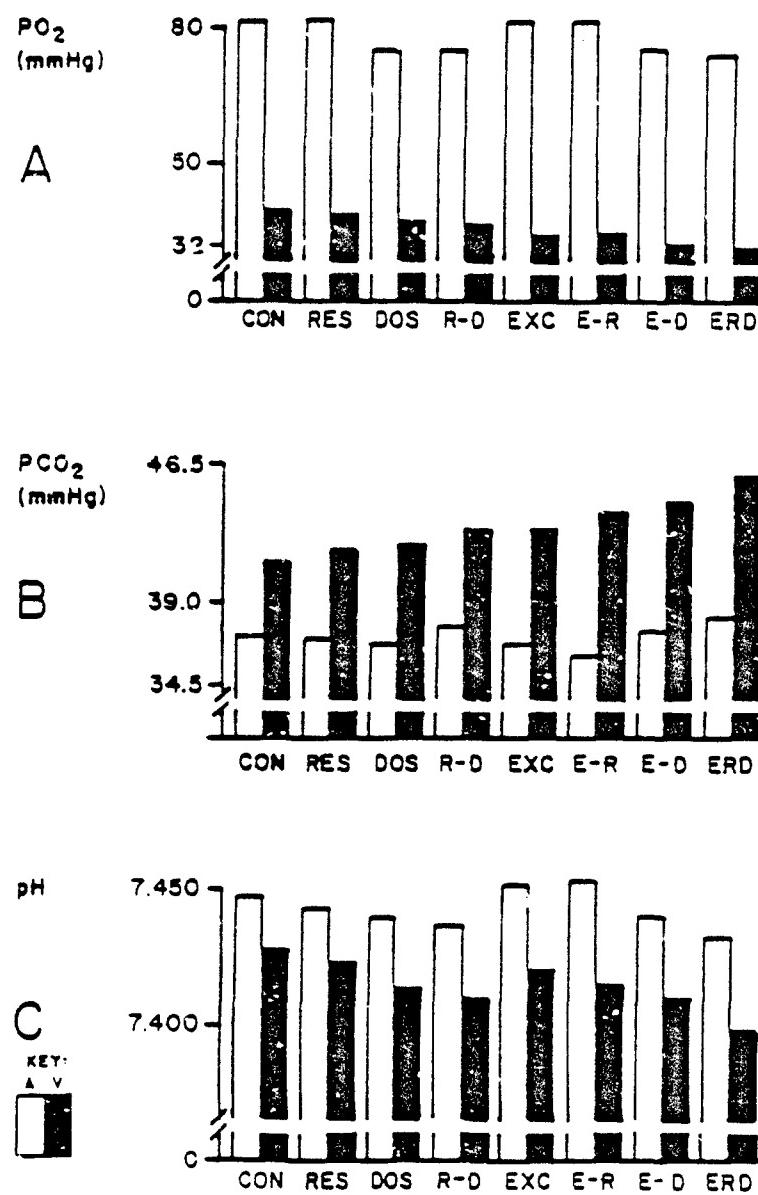
Figure III-J.   Alterations in Blood Gas Parameters, Simple

               Effects.

     Panel A presents mean arterial (open bars) and venous

(solid bars) blood oxygen tension (PO_2) values for each of

the eight experimental conditions.   Note that the dosed conditions

(DOS, RD: ED, ERD) always showed lower arterial and venous

values compared to the respective rest and exercise control

values (CON and EXC).   Panel B presents arterial and venous

CO_2 tensions (PCO_2).   Note the tendency toward elevated arterial

PCO_2 at rest and exercise when resistance is combined with

dose (RD and ERD, respectively).   Note also, the consistent

and roughly additive increases in venous PCO_2 as the load

conditions are applied.   Panel C presents arterial and venous

pH values.   Note the falling pH as the loads are applied.

FIGURE III J'
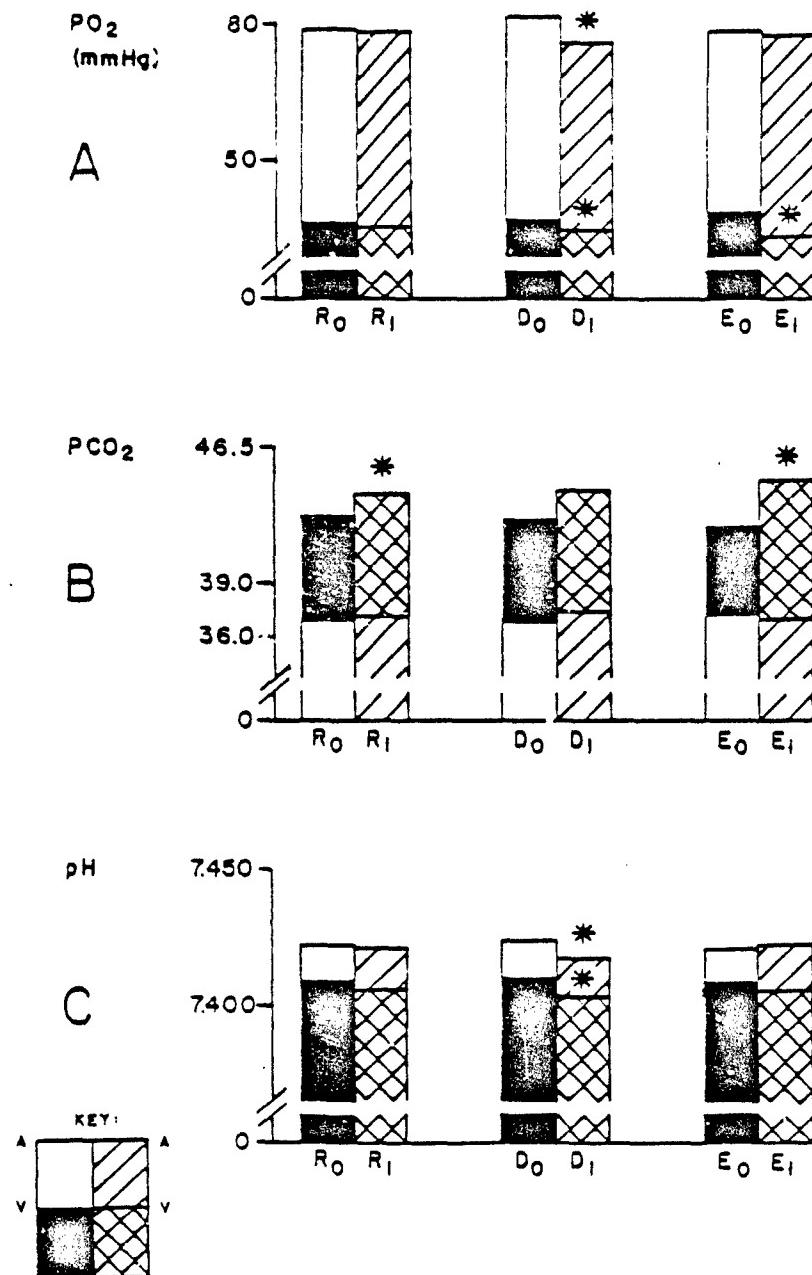
Figure III-J'.   Alterations in Blood Gas Parameters, Treatment
Main Effects.

Panel A depicts the main effects of resistance (R_1), dose
(D_1) and exercise (E_1) on arterial and venous PO_2 values. The
open and diagonally scored bars are arterial values, and
the solid and crosshatched bars are venous values. Panel B
and Panel C use the same notation for the treatment main effects
on PCO_2 and pH, respectively.

FIGURE Ⅲ K
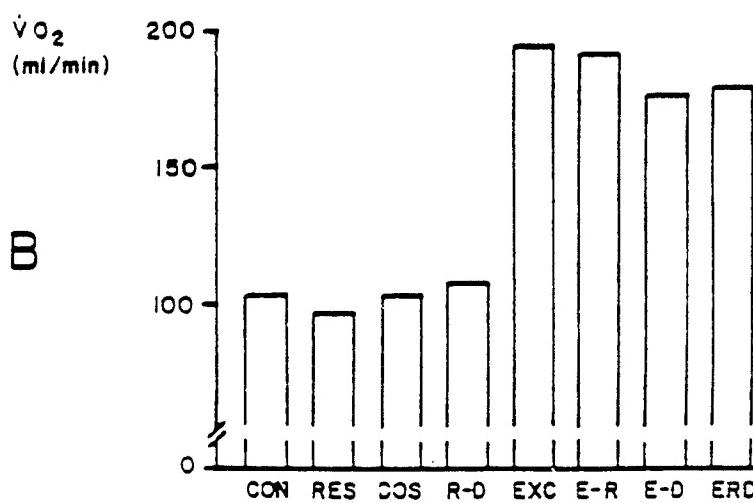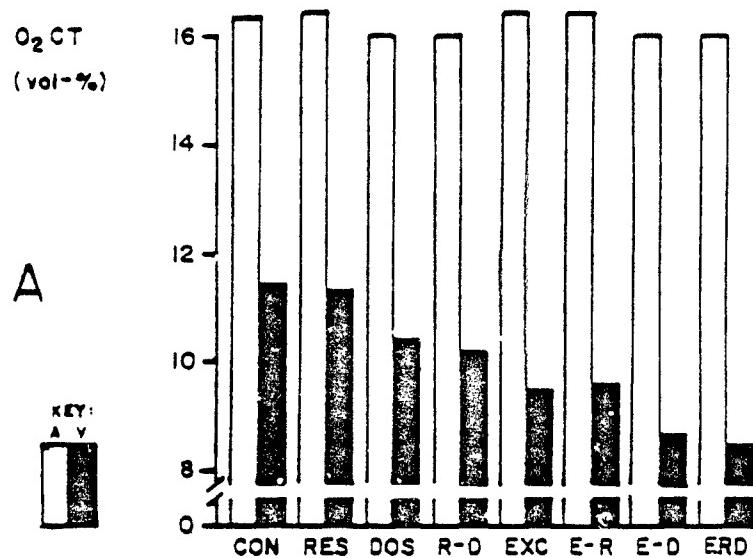
Figure III-K.   Alterations in Oxygen Content and Consumption,

Simple Effects.

Panel A presents arterial (open bars) and venous (solid bars)

oxygen content (O_2Ct) values as they change throughout the

eight experimental conditions. The difference between the

height of the arterial (open) bar and the venous (solid)

bar is the oxygen extraction, $(a-v)O_2Ct$. Panel B presents

the oxygen consumption ($\dot{V}O_2$) values as they are effected by

the experimental conditions. Note the diminished oxygen

consumption with resistance loading at rest (RES) and with

cholinesterase inhibition during exercise (E-D and ERD).

FIGURE III K'
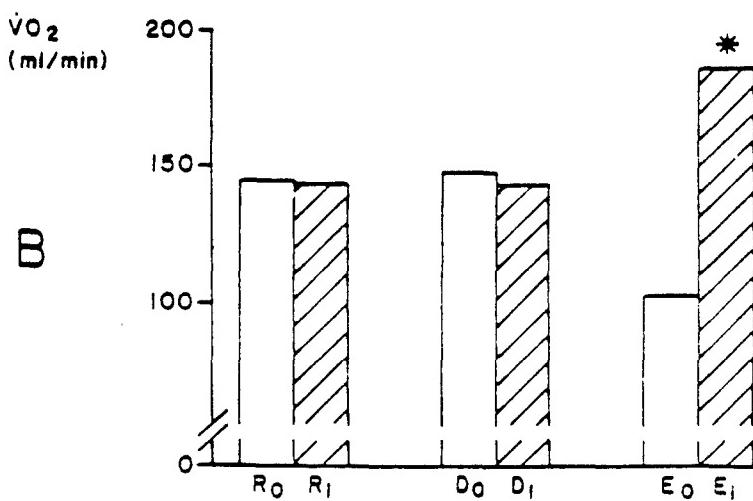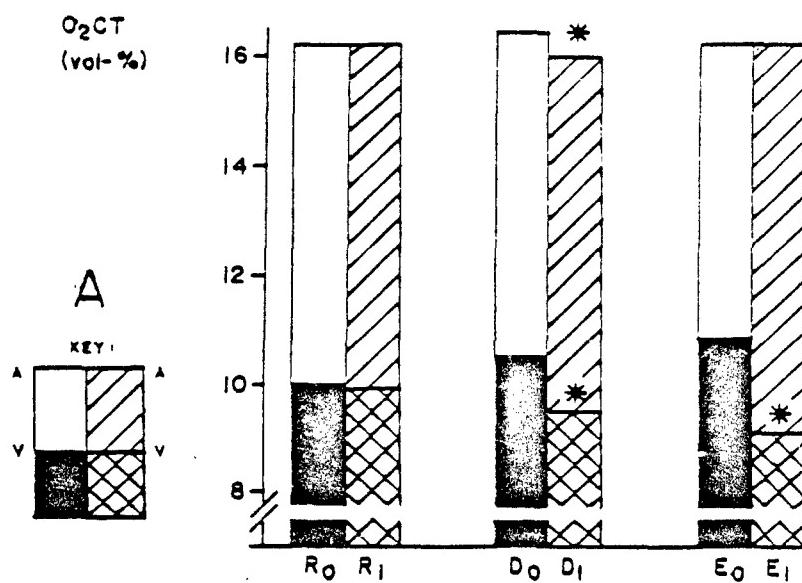
Figure III-K'.   Alterations in Oxygen Content and Consumption,

Treatment Main Effects.

Panel A depicts the main effects of the three treatment

variables on arterial and venous O_2Ct. The middle pair of

bars shows a significant decrease of oxygen content in

arterial and venous blood associated with dosing with

neostigmine (cholinesterase inhibition). Panel B depicts the

main treatment effects on $\dot{V}O_2$. The dose main effect is not

significant; however, a significant interaction was shown

between dose and exercise (D_1 x E_1). That interaction is

presented in Table III-C' and discussed in the text.

FIGURE III L
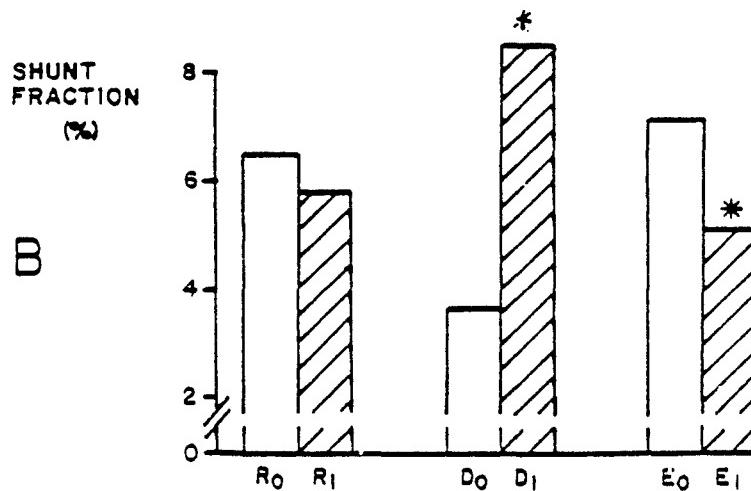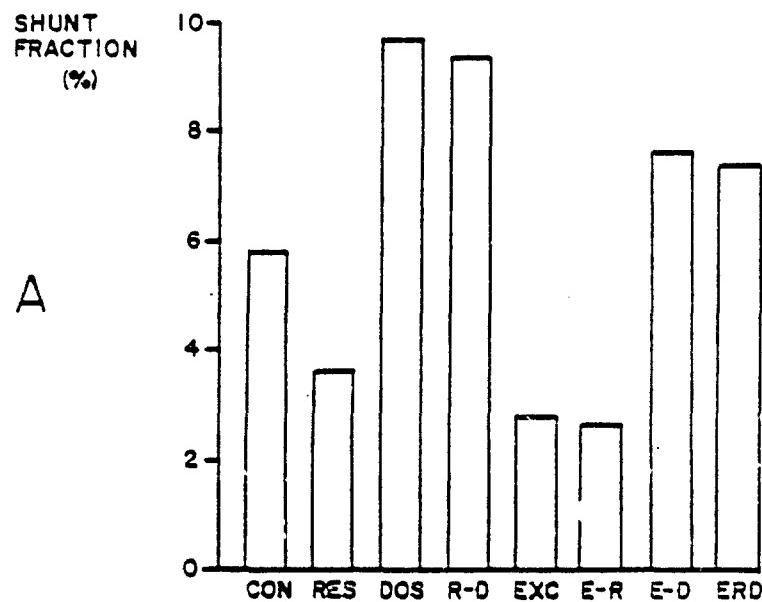
Figure III-L. Alterations in Shunt Fraction.

Panel A presents the simple effects of the eight treatment conditions on calculated shunt fraction (F_S). Note the consistent increase in F_S associated with the cholinesterase inhibited (dose) conditions. Panel B depicts the main effects of the three treatment variables on F_S. The middle pair of bars shows a large and highly significant increase associated with the neostigmine dose.

TABLE III C'. BLOOD GAS PARAMETERS, MAIN TREATMENT EFFECTS

PARAMETER	ABBREV	UNITS	Resistance			ChE Inhibition			Exercise			Interactions (see Footnotes)
			R_0		R_1	D_0		D_1	E_0		E_1	
Oxygen Tension, art.	P_aO_2	mmHg	78.6	NS	78.3	81.5	*	75.4	78.7	NS	78.2	None
Oxygen Tension, ven.	P_vO_2	mmHg	36.3	NS	35.9	37.3	*	34.9	38.6	*	33.6	None
Oxygen Content, art.	O_2Ct_a	Vol%	16.2	NS	16.2	16.4	*	16.0	16.2	NS	16.2	None
Oxygen Content, ven.	O_2Ct_v	Vol%	10.0	NS	9.9	10.5	*	9.5	10.8	*	9.1	None
A-V Difference	$(a-v)O_2Ct$	Vol%	6.2	NS	6.3	5.9	*	6.6	5.4	*	7.1	None
Oxygen Consumption	$\dot{V}O_2$	ml/min	145	NS	144	147	NS	143	103	*	186	E x D [1]
CO2 Tension, art.	P_aCO_2	mmHg	37.0	NS	37.3	36.8	NS	37.5	37.2	NS	37.1	None
CO2 Tension, ven.	P_vCO_2	mmHg	42.7	*	43.8	42.6	NS	44.0	42.2	*	44.4	None
pH, art.	pH_a		7.445	NS	7.441	7.449	*	7.437	7.441	NS	7.445	None
pH, ven.	pH_v		7.418	*	7.411	7.421	*	7.408	7.419	NS	7.411	None
Shunt Fraction	F_S	%	6.5	NS	5.8	5.7	*	8.5	7.1	*	5.1	None

(1) Oxygen Consumption

At rest (E0): $\frac{D_0}{100}$ NS $\frac{D_1}{106}$

At Exercise(E_1): 193 * 179; i.e.,ChE inhibition resulted in a significantly decreased $\dot{V}O_2$ during exercise, but had no significant effect at rest. In fact if anything, the $\dot{V}O_2$ increased under rest conditions due to ChE inhibition.

(d) There were no statistically significant interactions among the three treatment variables on P_aO_2. That is, the effect of cholinesterase inhibition in decreasing P_aO_2 was statistically the same whether resistance was present or absent and whether exercise was present or absent.

2. Venous Oxygen Tension (P_VO_2)(refer to Figure III-J and J^1, panel A).

(a) The computed main effect of inspiratory resistance loading on PO_2 was an insignificant decrease in tension from 36.3 to 35.9 mmHg (N.S.).

(b) The computed main effect of moderate cholinesterase inhibition on P_VO_2 was a significant decrease in tension from 37.3 mmHg to 34.9 mmHg after neostigmine was administered (p <.05). This fall in venous oxygen tension reflects not only greater tissue extraction of O_2, but also the lower arterial PO_2.

(c) The computed main effect of treadmill exercise on PO_2 was a significant fall in tension from 38.6 mmHg at rest to 33.6 mmHg during exercise (p <.05), indicating the greater extraction of O_2 by exercising muscle beds (since arterial PC_2 was not difference during exercise compared to the resting).

(d) There were no significant interactions among the three treatment variables on PO_2.

3. <u>Arterial Oxygen Content</u> (O_2C_a) (refer to Figure III-K and K', panel A).

(a) The computed main effect of inspiratory resistance loading on O_2C_a was negligible; it remained unchanged at 16.2 vol %.

(b) The computed main effect of moderate cholinesterase inhibition on O_2Ct_a was a significant decrease from 16.4 vol % to 16.0 vol % after neostigmine was administered (p <.05), suggesting areas of regional hypoventilation or shunt caused by cholinergic airway constriction or mucous blockage of some airways.

(c) The computed main effect of treadmill exercise on O_2Ct_a was negligible; the computed average content remained at 16.2 vol % at rest and during exercise.

(d) There were no statistically significant interactions among the three main treatment variables on O_2Ct_a.

4. <u>Venous Oxygen Content</u> (O_2Ct_v) (refer to Figures III-K and K[1], panel A).

(a) The computed main effect of inspiratory resistance loading on O_2Ct_v was an insignificantly small decrease from 10.0 to 9.9 vol % (N.S.).

(b) The computed main effect of moderate cholinesterase inhibition on O_2Ct_v was a significant decrease from 10.5 vol % before inhibition to 9.5 vol % after (p <.05), suggesting greater tissue extraction due to a smaller cardiac

output and increased work of breathing.

(c) The computed main effect of treadmill exercise on O_2Ct_v was a significant decrease from 10.8 vol % at rest to 9.1 vol % during exercise (p .05).

(d) There were no statistically significant interactions among the three main treatment variables on O_2Ct_v.

5. A-V Difference Oxygen Content $((a-v)O_2Ct)$ (refer to Figures III-K and K[1], panel A).

(a) The computed main effect of inspiratory resistance loading on a-v difference was a small insignificant increase from 6.2 to 6.3 vol % (N.S.).

(b) The computed main effect of moderate cholinesterase inhibition on a-v difference was a significant increase in O_2 extraction from 5.9 vol % to 6.6 vol % (p .05).

(c) The computed main effect of treadmill exercise on a-v difference was a significant increase in O_2 extraction from 5.4 vol % at rest to 7.1 vol % during exercise.

(d) There were no statistically significant interactions among the three main treatment variables on $(a-v) O_2Ct$.

6. Oxygen Consumption $(\dot{V}O_2)$ (refer to Figure III-K and K[1], panel B).

(a) The computed main effect of inspiratory resistance loading on $\dot{V}O_2$ was negligible (144.9 ml/min before loading, 144.1 ml/min after; N.S.).

(b) The computed main effect of cholinesterase inhibition on $\dot{V}O_2$ was a small and insignificant decrease from

147 ml/min to 143 ml/min after administration of neostigmine
(N.S.). Thus the increased tissue extraction induced by
the diminished cardiac output appears to have been adequate
to maintain O_2 consumption at predose levels, but in view of
increased demands by the breathing musculature, were main-
tenance of a prior level is surprising; moderate increases
would have been expected.

(c) The computed main effect of treadmill exercise
on $\dot{V}O_2$ was a significant increase from 103 ml/min at rest to
186 ml/min during exercise (p <.01).

(d) There was a significant interaction between
cholinesterase inhibition and exercise on $\dot{V}O_2$ (p <.05), therefore
the contrasting $\dot{V}O_2$ effects of cholinesterase inhibition at
rest and cholinesterase inhibition with exercise are presented
separately: At rest, cholinesterase inhibition increased oxygen
consumption from 100ml/min to 106 ml/min (N.S.). During exercise,
on the other hand, the same inhibition resulted in a decrease
in oxygen consumption from 193 ml/min before inhibition to
179 ml/min after administration of neostigmine (p <.05). This
fact is more in line with expectations in that during exercise
when tissue demands for O_2 are already increased, the further
increases induced by work of breathing demands in the face of
a decreased cardiac output are apparently not adequately com-
pensated for by greater tissue extraction. Redistribution of
blood may play a role.

7. <u>Arterial CO$_2$ Tension</u> (P_aCO_2)(refer to Figures III-J and J^1, panel B).

(a) The computed main effect of inspiratory resistance loading on P_aCO_2 was an insignificantly small increase (0.2 mmHg N.S.).

(b) The computed main effect of moderate cholinesterase inhibition on P_aCO_2 was an insignificantly small increase (0.8 mmHg, N.S.).

(c) The computed main effect of treadmill exercise on P_aCO_2 was negligible (N.S.).

(d) There were no statistically significant interactions among the three main treatment variables on O_2Ct_v.

8. <u>Venous CO$_2$ Tension</u> (P_vCO_2)(refer to Figures III-J and J^1, panel B).

(a) The computed main effect of inspiratory resistance loading on P_vCO_2 was a small but statistically significant increase from 42.7 mmHg to 43.8 mmHg after the introduction of the resistance (p <.05).

(b) The computed main effect of moderate cholinesterase inhibition on P_vCO_2 was an increase from 42.6 to 44.0 mmHg. This increase was not significant due to the large variability of response to neostigmine among the subject animals. Nevertheless, the trend is consistent and it is in agreement with calculated increases of muscular work of breathing during the inhibited experimental conditions.

(c) The computed main effect of treadmill exercise

on P_vCO_2 was a significant increase from 42.2 mmHg at rest
to 44.4 mmHg during exercise (p <.05).

(d) There were no statistically significant
inter? .ions among the three treatment variables on P_vCO_2,
that is to say that each treatment variable produced changes
in P_vCO_2 that were statistically consistent across all
combinations of treatment conditions; it should be recognized,
therefore, that the greater increase in P_vCO_2 occurs when
all three treatment variables are present (i.e., E*R*D condition,
P_vCO2 = 46.1 mmHg). This fact gives credibility to the additive
nature of the three treatment variables and belies any
statistically based synergism.

9. Arterial Blood pH (pH_a)(refer to Figures III-J
and J^1, panel C).

(a) The computed main effect of inspiratory re-
sistance loading on pH_a was an insignificant decrease from
7.445 to 7.441 (N.S.).

(b) The computed main effect of moderate cholin-
esterase inhibition on pH_a was a significant decrease from
7.449 to 7.437 after neostigmine administration (p <.05).

(c) The computed main effect of treadmill exer-
cise on pH_a was an insignificant increase from 7.441 at rest
to 7.445 during the exercise conditions (N.S.).

(d) There were no statistically significant
interacting among the three main treatment variables on PH_a.

10. Venous Blood pH (pH_v)(refer to Figures III-J

   and J^1, panel C).

(a) The computed main effect of inspiratory re-
sistance loading on pH_v was a small but significant decrease
from 7.418 to 7.411 after the introduction of resistance
($p < .03$).

(b) The computed main effect of moderate cholin-
esterase inhibition on pH_v was a significant decrease from
7.421 to 7.408 after administration of neostigmine ($p < .05$).

(c) The computed main effect of treadmill exer-
cise on pH_v was a small decrease from 7.419 at rest to 7.411
during the exercise conditions. This decrease does not quite
reach the statistical significance criterion.

(d) There were no statistically significant in-
teractions among the three treatment variables on pH_v, however,
as with P_vCO_2, the largest fall in venous pH occurs when all
three treatment stresses are present (i.e., E*R*D condition,
$pH_v = 7.398$).

11. Shunt Fraction (F_S)(refer to Figure III-L, panels

   A and B).

(a) The computed main effect of inspiratory re-
sistance loading on shunt fraction was a small decrease (absolute
decrease of 0.7%) that was not statistically significant.

(b) The computed main effect of moderate cholin-
esterase inhibition was a significant increase from 3.7%, averaged
among the uninhibited conditions, to 8.5% after the inhibition

was administered (p <.05). This "shunt fraction" (also
known as venous admixture) increase associated with cholin-
esterase inhibition indicates a change in the ventilation-
perfusion relationships of the lung and suggests that some
airways had become severely occluded, either from mucous
buildup, small airways smooth muscle constriction, competition
for space in the peribronchial sheath secondary to pulmonary
edema or any combination of these. The observed copious
amounts of respiratory tract mucous that was coughed up by
these animals gives credibility to at least that factor in
explaining these findings.

(c) The computed main effect of fixed treadmill
exercise on F_S was a significant but small decrease (from
7.1% to 5.1%; p <.05).

(d) There were no statistically significant in-
teractions among the three main treatment variables on the
shunt fraction calculations.

CHAPTER IV.   DISCUSSION

The objectives of this investigation were to character-
ize the mechanisms of, and quantify the cardiopulmonary
functional decrement associated with:

(1)   inspiratory resistance loading similar to that
produced by respiratory protective devices (gas masks), and

(2)   a moderate level of peripheral cholinesterase
inhibition (approximately 50%, as measured in whole blood).

The presence of interaction or additivity of these two
physiological loads was to be assessed when they were applied
simultaneously to an awake, instrumented, mongrel dog pre-
paration, both at rest, and during mild treadmill exercise.

The following discussion addresses interpretation of the
experimental results obtained in this study with application
to these objectives.

A.   Ventilatory Parameters

Resistance Loading. The inspiratory resistance load used
in these experiments had the consistent effect of lowering
minute ventilation (13% overall). The magnitude of this re-
sponse was similar whether at rest or exercise and whether the
animals had been pretreated with neostigmine or not. This
response was the combined result of a small but consistent
decrease in both breathing frequency and tidal volume. Generally,

however, an increase in inspiratory time relative to total time of the respiratory cycle (T_i/T_{tot}) accompanied inspiratory resistance loading to partially compensate for the less rapid filling of the lungs ($\dot{V}_{peak}$ fell significantly). This undoubtedly reflects the prolongation of electrical discharge (onset to peak) of the diaphragm, external intercostals, and accessory muscles and in the nerves supplying them, which is elicited by the Breuer-Hering inflation reflex (Cohen 1979; Siafakas et al, 1981). The resulting effect due to inspiratory resistance loading is that tidal volume has been partially defended but minute ventilation less well so. The resistance-induced increase in inspiratory tim_ (T_I) and the increased magnitude of inspiratory pleural pressure (PIP_{PL}) are reflected in the increased Pleural Pressure-Time Index (PPTI increased by 21% after the introduction of the resistance load). Thus, the oxygen cost of breathing (O_2CB) was increased by the resistance load even though the ventilatory output of the inspiratory musculature ($\dot{V}_{min}$) was decreased. Therefore, the constructed Breathing Effectiveness Index, the BEI ($\dot{V}_{min}$/PPTI, in $L \cdot min^{-1}$/mmHg) fell by 28% compared to the non-resistance loaded conditions. This decrease in the BEI is analagous to a relative decrease in efficiency (efficiency $= \dfrac{external\ work}{O_2CB}$) of the inspiratory muscles. This finding is in agreement with previous investigators who have found sharp decreases in respiratory efficiency due to added resistance loading (Robertson et al, 1977; Rochester and

TABLE IVA LUNG MECHANICS, HYPER-REACTIVE DOG

| PARAMETER	ABBREV.	UNITS	CON	RES	DOS	E*U	E*M	E*30	E*20	E*20-D
Frequency	f	breaths/min	20	18	24	22	30	29	30	28
Tidal Volume	V_T	ml	320	366	336	333	440	413	380	363
Minute Volume	V_{min}	l/min	6.40	6.95	9.62	8.16	13.20	11.58	11.40	10.16
Peak Flow	$\dot{Q}_{peak}$	l/min	26.4	22.0	30.6	27.5	37.6	28.8	32.3	28.8
Pleural Pressure, end-expiratory	EEP_{pl}	mmHg	-4.7	-3.0	-3.9	-5.0	-4.6	-4.3	-4.6	-7.2
Pleural Pressure, end-inspiratory	EIP_{pl}	mmHg	-9.8	-11.0	-20.1	-18.6	-11.8	-10.8	-19.8	-16.8
Pleural Pressure, peak inspiratory	PIP_{pl}	mmHg	-10.8	-13.2	-28.6	-24.0	-16.4	-17.4	-30.0	-35.2
Pleural Pressure, resistive component	ΔP_{pl}	mmHg	1.0	2.5	10.3	11.6	6.1	8.4	12.2	19.0
Lung Volume, at peak P_{pl}	V_L	ml	314	355	294	297	345	280	311	342
Dynamic Compliance	C_{dyn}	l/cmH2O	0.0461	0.0378	0.0152	0.0256	0.0448	0.0467	0.0184	0.0281
Airway Resistance	R_{aw}	cmH2O/l·sec⁻¹	3.1	8.3	27.5	31.0	13.2	18.3	30.8	39.5
Inspiratory Work Rate, resistive	W_{res}	kg·M/min	0.054	0.042	0.891	0.406	0.444	0.65	0.441	1.758
Inspiratory Work Rate, elastic	W_{el}	kg·M/min	0.646	0.697	1.344	1.019	1.512	1.267	1.972	1.730
Inspiratory Work Rate, total Dynamic	W_{dyn}	kg·M/min	0.700	0.779	2.237	1.725	1.956	1.920	3.415	3.489
Pleural Pressure Time Index	PPTI	mmHg	1.75	2.30	7.96	5.73	4.44	5.04	11.55	10.58
Breathing Effectiveness Index	BEI	($l·min^{-1}$/mmHg)	3.66	2.92	1.21	1.42	2.97	2.38	0.99	0.96
				REST						EXERCISE
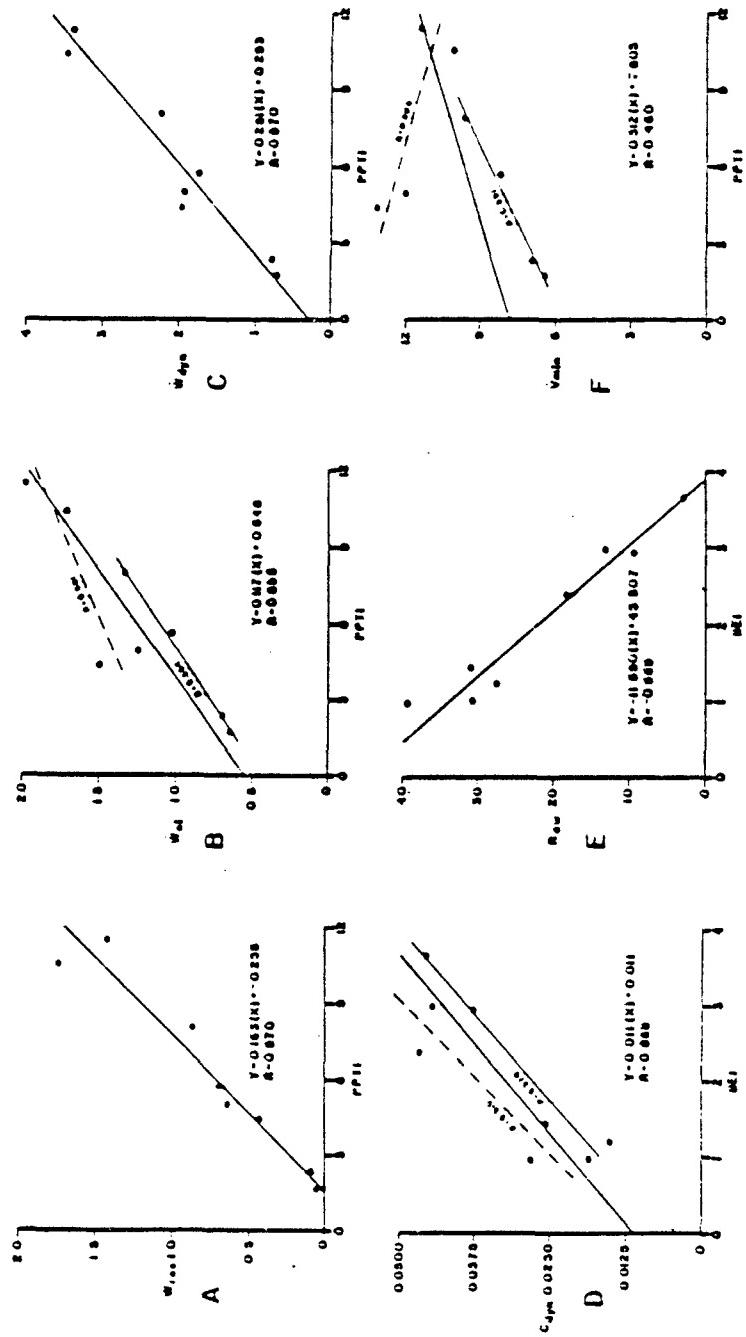
Figure IV-A.   Correlations of the PPTI and the BEI with

Pulmonary Mechanics (see text for discussion).

Bettini, 1976; McGregor and Becklake, 1961).

In a single dog, inspiratory work rat (W_{dyn}) wa, calculated dynamically with an adaption of the Campbell graphical method (Campbell, 1970; refer to Table IV-A).

It did not change significantly in response to resistance loading, yet the PPTI (reflecting the oxygen cost of breathing) increased by approximately 25% as com- pared to rest and exercise control values. This finding corroborates the idea that external work claculations generally underestimate the oxygen cost of breathing (O_2CB) during resistive loading that has been reported by numerous authors (Robertson et al, 1977; Rochester and Betrini, 1976).

The Breathing Effectiveness Index (which has the units of conductance: $L \cdot min^{-1}/mmHg$) consistently fell with the resistance loading, and this effect (average decrease in effectiveness = 36%, $p < .05$) was qualitatively and quan- titatively similar at rest and exercise, as well as before and after cholinesterase inhibition. This decrease in effectiveness, as previously allude to, is a product of either increased inspiratory flow resistance, increased elastic recoil (decreased compliance), or a component of both (impedance). In an effort to illustrate the relative contribution of each of these mechanical factors, dynamic compliance and airway resistance was cal- culated in a single dog with a graphical method (see Table IV) and the results correlated with the BEI (refer to Figure IV-A, panels D,E). Not surprisingly, this analysis

illustrates a very high degree of correlation (r = .97)
between the BEI and graphically calculated airways resis-
tance (R_{aw}), and a poorer correlation with changes in
dynamic compliance (r = .86), over the range of values
generated by this experimental protocol. However, if
one analyzes this relationship with the rest data (r = .97),
separately from the exercise data (r = .91) where lung
volumes (and thus recoil forces) are large, the correlation
with dynamic compliance improves, especially for the rest
data set. Nonetheless, both viscous and elastic forces
must be considered as components of this index even when
reflecting changes due to external resistance loading
since lung volume is not held constant, but this fact does
not detract from it's usefullness as an index of changes in
efficiency of the respiratory pump.

A similar analysis was performed correlating the
PPTI with total dynamic work rate of breathing ($\dot{W}_{dyn}$) and
its two components, work against flow resistance ($\dot{W}_{res}$)
and work against elastic recoil ($\dot{W}_{el}$). Figure IV-A, panels
A,B, and C depict these correlations. Again not surprisingly,
the PPTI shows a better correlation with $\dot{W}_{res}$ (r = .97)
than with $\dot{W}_{el}$ (r = .89). However, when the rest data is
evaluated separately (r = .99) from the exercise data
(r = .89), the correlation again improves for the rest values
of $\dot{W}_{el}$. Panel F, in Figure IV-A, merely shows the rather poor
correlation between minute ventilation and the PPTI (or by

induction, between muscular work of breathing and minute ventilation) across the experimental conditions, illustrating the fact that inspiratory efficiency does indeed change with various patterns of breathing and therefore ventilatory output ($\dot{V}_{min}$) per se is not a good index of the energy demands made upon the respiratory musculature.

This graphical analysis gives considerable credibility to the use of PPTI and the BEI as indices of changes in the oxygen cost of breathing, and of changes in inspiratory muscle efficiency respectively, at least for the range of ventilatory patterns encountered in this study.

In summary then, the inspiratory resistance load in these experiments increased the inspiratory muscular work of breathing and decreased the efficiency of the ventilatory muscular apparatus. The actual physiological stress then, is a result of not only the external work increase induced by the resistance load but also a superimposed diminution of muscular efficiency as well. In an otherwise unstressed, healthy animal this load alone does not appear to have severe respiratory consequences. On the other hand, the application of additional loads from increasing levels of exercise and/or cholinesterase inhibition have resulted in additive decrements of ventilatory function as indexed by the PPTI and the BEI (refer to Figure III-C, and Table III-A). Projection of this trend to higher levels of exercise and/or more severe

cholinesterase inhibition should undoubtedly result in arrival at a point where fatigue of the respiratory muscles will occur leading to acute respiratory failure, that is, when energy demands of the inspiratory muscles exceed supplies.

Cholinesterase Inhibition. The outward muscarinic and nicotinic symptomotology observed from the intravenous administration of neostigmine (0.025 mg/kg) was consistent and predictable in six out of the seven dogs in this study. Within 2-3 minutes after dosing, salivation, lacrimation, mild coughing and gagging, defecation and/or urination, and minor muscle fasciculations were observed. The salivation and coughing response persisted throughout the experimental period but was not severe enough to prevent accomplishment of the protocol (or interfere with the recordings or data collection, except for occasional expectoration of a clear jelly-like mucous). In one of the dogs, however, (referred to as the "hyper-reactive dog", Table III-D) the immediate respiratory symptoms were dramatically more severe. The inspiratory resistive work rate ($\dot{W}_{res}$) increased 18-fold within minutes of the administration of the drug; this was the combined result of a cholinergically stimulated increase in ventilation ($\dot{V}_{min}$ increased by 50% at rest) and greatly increased airway resistance (R_{aw} increased 9-fold at rest). The muscular work of breathing, indexed by the PPTI, increased by 450%.

To put this large increase in work of breathing into
perspective, the external resistance of the respirator mask
(RES) only increased the PPTI by 36% at rest. It should be
further emphasized here that this particular dog's cholin-
esterase inhibition-reactivation profile with time
was not different from that of the other dogs in the study,
and the observed responses to the drug were replicated on
4 separate days with identical results. We may conclude,
therefore, that this dog was indeed an example of airways
hyper-reactivity to neostigmine, resembling an asthmatic
attack. The realization of the presence of large numbers
of asthmatics (diagnosed or sub-clinical) in the human
population should be a matter of concern for those who
would use cholinesterase inhibitors clinically, prophyl-
actically, or environmentally, because without immediate
supportive care this hypersensitive "subject" would get
into difficulties.

Neostigmine admistration increased breathing frequency
significantly in all dogs and in all treatment conditions
(Table III-A). This increase suggests a stimulant effect
on the central respiratory center. Yet Schaumann (1959)
and others have shown that neostigmine does not (except in
quite high concentrations) pass the blood-brain barrier.
Therefore, it is likely that this central respiratory
stimulation is reflexly indcued by afferents from perpheral
chemoreceptors. This excitatory activity is probably
localized at the ganglionic level, since Weinstock (1981)

showed that the neostigmine excitatory response in rabbits could be blocked by hexamethonium, a ganglionic blocking agent. It is likely that an additional stimulant effect acts indirectly at the chemosensitive area of the receptors themselves, since small increases in arterial PCO_2 (and pH) and significant decreases in PO_2 were also observed after neostigmine administration, thereby raising the chemoreceptor afferent activity through "normal" stimulation.

The neostigmine-induced changes in minute volume ($\dot{V}_{min}$) are less consistent. At rest, $\dot{V}_{min}$ was increased by 21%, while during the exercise conditions, $\dot{V}_{min}$ fell by 4%. The difference between the rest and exercise response was statistically significant (p < .05). The reasons for this disparity are not totally clear, but we can apply Milic-Emili's method of analysis of the breathing cycle (1976) to the data in an attempt to analyze the relative contribution of neuromuscular drive ($V_T T_I$) in distinction from the timing mechanism (T_I/T_{TOT}), which has been held to be related to the active impedance of the respiratory system. Milic-Emili's equation is:

$$\dot{V}_{min} = \underset{\text{(mean inspiratory flow)}}{V_T/T_I} \qquad X \quad \underset{\text{(duty cycle)}}{T_I/T_{TOT}}$$

and substituting from the experimental group data:

At Rest.

      Control: 12.4 L/min = 306ml/.68 sec   X 0.46
      (CON)   (27.0 L/min)

      Neostigmine: 15.2 L/min = 275ml/.51sec   X 0.47
      (DOS)   (32.4 L/min)

During Exercise,

      Control: 22.1 L/min = 419ml/.57sec   X 0.50
      (EXC)   (44.1 L/min)

      Neostigmine: 20.9 L/min = 342ml/.49sec   X 0.50
      (E*D)   (41.9 L/min)

At rest, this analysis clearly shows that inspiratory drive (V_T/T_I) rises with neostigmine administration mainly due to a sharp increase in frequency which sortens inspiratory time. This more than compensates for a decreased tidal volume, with the result that minute ventilation is increased 23% at rest. The observed fail in tidal volume may reflect a cholinergically mediated "early" stimulation of the Breuer-Hering inflation reflex, which has the normal role of stopping inspiration when an adequate lung volume is reached. In contrast, during exercise the increase in frequency ind···· ·d by neostigmine (as reflected by a shortened T_I) is less than adequate to overcome a now larger shortfall of tidal volume, resulting in a somewhat decreased minute ventilation (approximately 5%). It is reasonable to interpret this disparity on the basis of pre-existing afferent neural

traffic from the chemoreceptor and the Breuer-Hering stretch
receptor pathways. That is, at rest and afferent traffic
is relatively low and any prolonged ganglionic excitatory
effects would be readily apparent. During exercise on
the other hand, where receptor afferents are firing with
greater temporal and spatial facilitation (increased frequency
and number of neurons firing) a similar cholinergic en-
hancement might not elicit as dramatic an effect. In other
words since frequency is already elevated due to the exercise
stimulation, a further stimulation by a peripheral afferent
might not produce an equivalent increase in the centrally
mediated rate of firing of the phrenic nerve efferents.
This could explain the smaller increase in breathing
frequency mediated by chemoreceptor afferents in response to
the neostigmine challeng: during exercise compared to that
at rest. However, there is no reason, as yet, to believe
that different receptor reflexes respond to a given degree
of peripheral cholinesterase inhibition in a predictable way
and hypotheses concerning their behavior are purely speculative
and await further testing.

Another explanation of the differentially decreasing tidal
volume has merit: The decrease may be due to a simple mechanical
effect of increased airway resistance induced by cholinergic
bronchoconstriction, a well-documented muscarinic response.
As already shown, inspiratory resistance loading (externally,
by a respiratory mask) decreased tidal volume and minute

ventilation at rest and during exercise. There is no reason

to suggest that this response would be any different if

the resistance was of physiological origin (bronchocon-

striction, airway secretions, etc.). Indeed, the PPTI,

which reflects the muscular work of breathing, rose

significantly after neostigmine; and the BEI, indexing the

efficiency of the inspiratory maneuver, fell significantly;

the percent chan-e was greater during exercise than at

rest (though not significantly so). Thus we might

conclude that neostigmine-induced bronchoconstriction in-

creases the muscular work of breathing by increasing

airways impedance and ventilatory drive at rest; while

during exercise the higher ventilatory flow levels are not

sustained because increased airway impedance delays filling

of the lungs by increasing the phase lag (time constant) of

the respiratory "pump". The non-linear nature of resistance to

flow in the cholinergically constricted large airways could

readily account for the observed increase of the muscular work

of breathing (PPTI) in the face of diminished minute ven-

tilation during exercise. Since, as has already been discussed,

increases of inspiratory flow resistance decrease the efficiency

of the ventilatory muscular "pump" and adds to the metabolic

burden of the respiratory muscles. This latter fact is

corroborated by the BEI which fell significantly after

neostigmine administration (32%, Table III-A; and Figure III-C

and III-C[1], panel B).

Figure IV-B graphically depicts the changing relationship between inspired minute ventilation ($\dot{V}_{min}$) and the index of changes in musc ar work of inspiring (PPTI, i.e., the oxygen cost of inspiration) throughout the eight experimental challenge conditions. As defined previously, the slopes of the eight lines express the relative effectiveness of the inspiratory muscles in providing ventilation to the lung (i.e., the BEI) during the corresponding eight treatment conditions. The concept of additivity of the stress consequent to application of the three main treatment challenges is readily illustrated. For example, the slope of the solid line labeled CON is the BEI value for the rest control condition (i.e., standing, breathing quietly). This value (8.61 ± 1.57 L·min^{-1}/mmHg) is the highest effectiveness (i.e., efficiency) rating of all the observed treatment conditions. When resistance loading was added, labeled RES, the slope (BEI) fell to 5.75 ± 0.78 L·min^{-1}/mmHg, a relative drop-off in efficiency of 33%. With the resistance removed but after neostigmine administration at rest (labeled DOS), the BEI dropped to 5.19 ± 2.11 L·min^{-1}/mmHg. This particular condition's response was accompanied by the largest variability in the magnitude of change; however, this was due exclusively to the very large respiratory response of a single "hyperreactive" dog. It should be emphasized that all the dogs in this study, and every replication of the experimental trials

Figure IV B INSPIRATORY EFFICIENCY, THE BREATHING EFFECTIVENESS INDEX

Figure IV-3.   Inspiratory Efficiency, the Breathing

Effectiveness Index.

The solid and dashed lines in this figure represent
the relationship of minute ventilation (output of the
respiratory pump) with the pleural pressure-time index
(an index of the oxygen cost of breathing), at rest and
during exercise, respectively. The slope of each line
represents the Breathing Effectiveness Index (indexing
changes in efficiency of the inspiratory muscles in
generating minute flow, V_{min}). Note the additive decrement
in efficiency as each experimental load is added in turn.

within each dog, responded in the same direction of change
in the BEI (as well as the PPTI) to each of the eight
experimental treatment conditions; but all 4 conditions
where neostigmine had been administered have response
values that are somewhat shifted in magnitude toward the
response of the "hyper-reactive" dog. On the other hand,
the interrelationships of the treatment responses are not
different for that dog and it's large respiratory response
does not detract from the statistical consistency of the
group data and inclusion in the data set does not generate
any false physiological conclusions.

Nevertheless, when both resistance loading and cholin-
esterase inhibition were combined (labeled R*D), the BEI
fell further than either individual response, and furthermore
the combined effect was roughly additive (the R*D fell to
4.76 ± 1.07 L·min^{-1}/mmHg). A very similar pattern of
additivity is illustrated in the exercise BEI values (dotted
lines in Figure IV-B). The exercise control value (labeled
EXC) was $6.02 \pm .97$ L·min^{-1}/mmHg; the addition of resistance
during exercise (E*R) resulted in a 40% decrement in the BEI
($3.62 \pm .43$ L·min^{-1}/mmHg); neostigmine during exercise (E*D)
produced a similar fall in inspiratory efficiency (to
$3.67 \pm .90$ L·min^{-1}/mmHg). Both resistance and dosage with
neostigmine cominbed during exercise (ERD) produced an
approximately additive drop-off in efficiency to $2.81 \pm .55$ L·min^{-1}/
mmHg.

Not only are the resistance and neostigmine challenges approximately additive during rest and exercise in lowering inspiratory efficiency, but the effect of exercise itself, alone, and in combination with the other two challenges, produces an additive fall in inspiratory efficiency. At first glance this finding seems surprising, since it is widely held that airway resistance decreases during exercise, presumably due to an adrenergic airways dilatory effect (Cabezas et al, 1971; Kamon and Daniel, 1980; Suzuki et al, 1976). On the other hand in normal lungs the elastic and chest wall forces are proportionately much larger than the viscous flow-resistive forces that must be overcome to inflate the lungs during inspiration. Consequently, with the increased lung volumes associated with exercise (V_T increased with exercise by approximately 37% in these dogs, $p < .01$), coupled with the exercise-induced increase in breathing frequency, it is apparent that the muscular "work" rate of breathing should rise (PPTI rose by 153% from exercise alone) proportionately more than the external work output ($\dot{V}_{min}$ rose with exercise by only 76%).

Thus, this rather novel, but very reproducible approach to analyzing respiratory muscle energy demand (PPTI) relative to output ($\dot{V}_{min}$) of the respiratory "pump" can be used to interpret changed ventilatory function in terms of efficiency (or effectiveness if one prefers) of the inspiratory muscular apparatus. Further, its use in assessing the relative degree

of stress associated with respiratory system challenges or
combinations of challenges, such as resistance loading,
cholinesterase inhibition, and exercise. Unfortunately,
the respiratory data gathered in this investigation do
not allow the use of the PPTI or the BEI to predict
respiratory muscle fatigue, since no challenge or com-
bination of challenges produced positive, direct evidence
of development of fatigue. However, failure of the in-
spiratory muscles to produce adequate subatmospheric
pleural pressure during inspiration to drive "adequate"
ventilatory flows in strongly suggested in the analysis of
blood gas data that follows. There is no question that
future work with more severe loading, ChE inhibition, or
exercise conditions will allow correlations of the PPTI and
BEI with more direct measures of fatigue-induced respiratory
failure, and will thus allow their use as predictive in-
struments, forecasting incipient respiratory muscle failure.

B.   Circulatory Parameters

Resistance Loading. At rest the inspiratory resistance
imposed by the respirator mask produced a small but very
consistent decrease in heart rate (6 beats/min) and an
associated small decrease in cardiac output (128 ml). Neither
of these small changes were statistically significant, but
they were so consistent in direction that they merit brief

discussion. Schrijen et al (1975) reported significant decrease in heart rate and aortic pressure following spontaneous deep inspirations (i.e., sighs; when pleural pressure descends significantly below normal inspiratory maneuver levels), and this decrease was ascribed to an autonomic stretch reflex mediated by the abnormally sub-atmospheric pleural pressure. In the present study, both peak and mean inspiratory pleural pressure were significantly lower after inspiratory resistance loading with the result that all the transmural atrial and vascular pressures were higher, that is more stretched. Indeed, several investigators have reported that increased stretch of the atrial receptors can indeed induce a bradycardia response, but this response may be reversed depending on the underlying level of sympathetic tone (Edis et al, 1970; Aviado et al, 1955). In another study, stretch of the aortic wall by increased transmural pressures was also demonstrated to produce bradycardia by exciting the aortic baroreceptors (Fitzgerald, 1981).

In these experiments during exercise, when sympathetic tone to the heart is increased and metabolic demands of the tissues are greater, the bradycardia tendency elicited by resistance loading was lost (refer to Table III-3). As a result, the overall main effect of this inspiratory resistance load (7.5 cmH_2O) $L \cdot sec^{-1}$, measured at 50 $L \cdot min^{-1}$ flow) on heart rate and cardiac output was small and not apparently of pathophysiological significance in this group of experimental

animals. On the other hand, the mean transmural aortic pressure measured at end-diastole ($\overline{TMP}_{ao}$, diast., the effective afterload to left ventricular ejection) rose significantly by approximately 5 mmHg during the exercise-resistance loading experimental conditions, while left ventricular filling pressure (i.e., transmural left atrial pressure, TMP_{LA}) remained unchanged. This translates into an increase in left ventricular work rate ($\dot{W}_{LV}$) which places an additional metabolic load on the myocardium ($\dot{W}_{LV}$ rose by 9% during resistance loading with exercise). Again, this amount of cardiac stress is of questionable physiological significance for healthy subjects with no other cardiopulmonary impairment, but those with underlying chronic disease may well be placed in jeopardy especially if exercise stresses are added. Furthermore, in evaluating the integrated effects of resistance loading on the circulation one must also include the added muscular work of breathing and it's associated metabolic demands that ultimately the circulation must satisfy. The PPTI rose by 21% overall due to resistance loading and since the muscular work of breathing normally only represents from 2-10% of the normal whole body energy expenditure this increase is a small load on the whole body circulation. However it is not a small load in terms of the specific perfusion of the inspiratory musculature which has been proven to be perfusion limited in the onset of respiratory muscle fatigue (Robertson et al.,1977). Nevertheless, this is the first hard physiological data,

that I am aware of, that may be used as the basis for exclusion of certain cardiovascular-impaired subjects from respirator use on the job.

Cholinesterase Inhibition. The single most important effect of neostigmine administration in these experiments was the reduction of cardiac output. Mean arterial pressure fell commensurate with this reduction in flow and pulmonary artery pressure was unchanged; thus no evidence of a neostigmine pressor response was observed. This finding does not contradict the observations of Levy and Ahlquist (1962) and of Hilton (1968) who observed an increased blood pressure response in anesthetized dogs treated with neostigmine only after ganglionic (nicotinic) blockade. These investigators worked with anesthetized, ganglion blocked animals and their results were not applicable to an awake, unanesthetized preparation.

The fall in cardiac output was the combined result of a decrease in heart rate and a diminution of myocardial contractile ability mediated by a neostigmine-induced elevation of cholinergic vagal tone on the heart. The hemodynamic evidence for this conclusion is convincing. After the neostigmine dose transmural left atrial pressure (TMP_{LA}) rose significantly; at the same time transmural aortic pressure measured at end-diastole (TMP_{ao},diast.) fell significantly, while stroke volume (SV) rose only very slightly. In other words, filling pressure for the left ventricle rose (by approximately 3 mmg) even though afterload to left ventricular

ejection was considerably diminished (by approximately 7 mmHg). Thus the heart was unable to keep pace with pumping out its venous return without elevating it's filling pressures (TMP_{RA} and TMP_{LA} both rose significantly and are suggestive of congestion of blood in the heart and lungs). And since this congestion was not induced by an increase in afterload, the inescapable conclusion to be drawn is that both heart rate and myocardial contractility (chronotropic and inotropic responses, respectively) were diminished; because a slowed heart rate alone would be compensated for by an increased stroke volume through the "Frank-Starling" mechanism (Starling,1918). That is, in a normally functioning heart a slowed rate allows greater filling time; and thus volume of the ventricle prior to systolic contraction would be enlarged. The increased diastolic volume translates into greater length of the myocardial muscle fibers and greater length translates into greater force of contraction which would empty the ventricles during systole. Thus, in the normal uncompromised heart a mere reduction in heart rate would be compensated for by increased diastolic volume and ejection of that volume during systole, thereby maintaining the cardiac output. This compensatory increase of stroke volume is conspicuously inadequate to maintain the cardiac output after neostigmine administration in these dogs and is the basis of the negative inotropic response conclusion. The fact that systemic tissue

extraction of oxygen and CO_2 production were both sig-
nificantly increased with cholinesterase inhibition
and arterial PO_2 was significantly reduced (i.e., the
chemoreceptor drive for cardiac output was elevated)
supports this conclusion.

The fall in cardiac output associated with neostigmine
administration was observed to be significantly different
in its magnitude during exercise as compared to the rest
conditions ($\dot{Q}_{ao}$ fell by 122 ml/min at rest and by 278 ml/min
during exercise; 6% and 9% respectively). This exaggerated
negative effect on cardiac function may be an example of
what Levy (1977) has termed "accentuated antagonism". The
classic example of this effect in the literature was the
work reported by Hollenberg et al (1965) who found that
acetylcholine infused into a coronary artery in dogs had
only a slight negative inotropic effect on the ventricular
myocardium. However, if the same infusion was administered
during increased activity of sympathetic nerves to the heart
or during a constant infusion of norepinephrine, the myo-
cardial depression produced by acetylcholine was much more
pronounced. Similar interactions between the sympathetic
and parasympathetic controls of the heart have been shown
by numerous authors (Levy et al, 1966, 1971); Dempsey and
Cooper 1969; Levy and Zieske 1969; Bailey et al, 1979).
Certainly the level of neural sympathetic tone to the heart
had been increased by the treadmill exercise and undoubtedly

humoral levels of norepinephrine released by the adrenals were elevated as well. Thus it is reasonable to invoke this interactive mechanism as an explanation of the post-neostigmine hemodynamic data, since Loffelholz and Muscholl (1969) have published convincing evidence of muscarinic inhibition of norepinephrine release from sympathetic nerve fibers to the heart and further showed that the inhibition could be blocked entirely by atropine. This apparent interaction of cholinesterase inhibition with exercise, inducing non-linear diminution of cardiac output, is impor-tant for at least two reasons. First, if the trend were to continue, with higher levels of sympathetic activity induced by exercise it is reasonable to suggest that severe energy deficits at various tissue levels would ensue since the cardiac output would be falling at just such a time when rising metabolic needs, especially of the heart and ventilatory musculature, are rapidly increasing (left ventricular work rate, $\dot{W}_{LV}$, rose by 60% and the Pleural Pressure - Time Index, PPTI, rose by 130% from the treadmill exercise alone). Secondly, the inability of the circulation to adequately clear metabolic byproducts such as CO_2 and lactate would further compromise the ability of these two all-important muscular "pump" systems to continue their respective outputs effectively ($\dot{Q}_{ao}$ and $\dot{V}_{min}$). Both the heart and the respiratory muscles have been shown to be perfusion limited in their contractile abilities in that neither has a large

capicity for anaerobic work (George et al, 1961; Kar and

Pearson, 1963) and both increase their rates of perfusion

in response to increased work loads (Rochester and

Bettini, 1976). Aubier et al (1981) demonstrated respir-

atory muscle fatigue developing as a direct result of

tamponade-induced decreases in cardiac output. They found

that under these conditions the work of breathing was

substantially increased due to hyperventilation elicited by

acidemia (decreased arterial pH) and hypoxemia (decreased

arterial PO_2) and alterations in pulmonary mechanics secondary

to pulmonary vascular congestion. They further showed that

in consequence to the decreased cardiac output, respiratory

muscle blood flow was limited to levels less than those required

by the increased work of breathing, leading to respiratory

muscle fatigue and respiratory failure due to an impairment of

the contractile processes of the muscles. As a close parallel

in the present experiments, the previously described changes

in ventilatory mechanics that were induced by cholinesterase

inhibition or external resistance loading also increase the

work of breathing. The observed fall in cardiac output in-

duced by cholinesterase inhibition (though smaller in magnitude

than Aubier's tamponade-induced 35% levels) must certainly

diminish the amount of perfusion available to the respiratory

muscles (as well as to the heart), although local vasodilatory

mechanisms may mitigate this effect within certain limits.

The following discussion of the blood gas data obtained in these experiments will demonstrate the tendency toward the development of respiratory fatigue due to the "additive" challenge of resistance loading, cholinesterase inhibition and exercise.

C. Blood Gases

Resistance Loading. The addition of an inspiratory resistance to the respirator mask - pneumotachometer system used to measure ventilation dynamics had not significant effect on arterial blood gas parameters. This observation demonstrates that under the challenge conditions imposed during this protocol the resistance load per se did not impair pulmonary gas exchange to any measureable extent. On the other hand, both venous PCO_2 and venous pH were significantly altered (PCO_2 rose by 1.1 mmHg overall, and pH fell a commensurate amount from 7.418 to 7.411). These venous changes, though small in physiological terms, probably indicate the extra increment of muscular work (i.e., energy metabolism) demanded of the inspiratory muscles by the imposition of this load. If one assumes that this increase in mixed venous PCO_2 was produced by the admixture of only 1 to 5% fo the cardiac output, the fraction which perfuses the inspiratory muscles (from the data of Robertson et al, 1976), it is apparent that a much larger CO_2 production and thus metabolic expenditure must have

been demanded of the inspiratory muscles during the re-
sistance loading. This conclusion appears reasonable
since the cardiac output overall had not fallen in response
to resistance loading, and if anything, perfusion to the
respiratory muscles would have increased considerably,
as demonstrated in the diaphragm of the dog by Robertson
et al (1977), Rochester and Bettini (1976) and several others.
The increased venous PCO_2 values were measured in blood
samples that were drawn within three minutes of the imposition
of the resistance load and all other external conditions held
constant; therefore other changes in whole body metabolism
that could account for an increased venous PCO_2 are unlikely.
An insignificant, but very consistent, small drop in oxygen
consumption was observed after the imposition of the resistance
load. This same but larger fall in $\dot{V}O_2$ was observed by
Hanson et al (1961) in humans with externally applied breathing
resistance. They concluded that this fall was associated with
their observed significantly decreased minute ventilation.
This conclusion appears to be valid to explain the present data
as well.

Cholinesterase Inhibition. Arterial PO_2 and oxygen content
fell significantly after cholinesterase inhibition while oxygen
extraction by the tissues increased (as reflected in the
significantly larger a-v difference in oxygen content). Arterial
and venous pH were both significantly more acid, while arterial
and venous PCO_2 values were somewhat elevated but not enough

to reach the significance level. To gain further insight
into the cause of the fall in arterial PO_2 with ChE
inhibition, physiological shunt was calculated using
the shunt equation (see Chapter II, Methods). The
important result was that shunt fraction increased sig-
nificantly after ChE inhibition and the relative change was
greater during exercise (from 4.7% to 9.5% at rest; from
2.7% to 7.5% during exercise), whereas the absolute change
induced by ChE inhibition was the same, about 5% increase in
shunt fraction. The implication of this finding is that after
neostigmine administration, in spite of stimulated minute
ventilation, certain areas of the lung were being hypo-
ventilated. That is, the alveoli were receiving perfusion but
were not adequately ventilated to achieve equilibrated gas
exchange; thus the arterial blood gases reflect this so-called
"venous admixture" of blood that is low in O_2. It is not
difficult to explain this finding, it probably results from one
or any combination of three possible mechanisms; (1) increased
peripheral airways resistance caused by pulmonary congestion
and/or competition for space between arteries and small
airways secondary to the neostigmine-induced increase in left
atrial pressure (Hogg et al, 1972). This mechanism could have
the effect of lowering the ventilation-perfusion ($\dot{V}/\dot{Q}$) ratio
in the areas so effected, producing "shunt-like" blood gases;
(2) A simpler and equally likely mechanism could have been the
occlusion of some airways or even complete segments of lung

by the accumulation of secretions of copious amounts of
mucous that were observed after neostigmine administration.
The "shunt" result would be the same, and the resulting
hypoxemia contributes to the developing metabolic stress
(3). The increase in pulmonary vascular blood volume secondary
to the increased left atrial pressures after neostigmine,
may have induced a cardiogenic pulmonary edema-like
syndrome. If this were so, some alveoli, starting in the
lower-most regions of the lung could begin to fill with
fluid thereby preventing gas exchange; in other words,
another shunt condition would exist in these perfused but
unventilated alveoli. Quantification of the magnitude of
each of these mechanisms awaits future investigation, but
considering the hydrostatic gradient from the left atrium
to the lowermost lobes of the lung, it is quite likely that
the local capillary pressures there (20-25 mmHg) would have
been sufficient to produce edematous effusion into some alveoli.

Oxygen consumption ($\dot{V}O_2$) was calculated with the Fick
Principle (Rahn and Fenn, 1955) utilizing directly measured
cardiac output times a-v oxygen difference ($\dot{V}O_2 = \dot{Q}_{ao} \times (a-v)$
$O_2 \ C_t \times 100^{-1}$). The major significant finding was that $\dot{V}O_2$
fell during exercise after cholinesterase inhibition
while during the rest protocol it had remained unchanged.
This result is especially surprising in light of the 46%
increase in muscular work of breathing (PPTI) that was found
to occur with cholinesterase inhibition (refer to Figure IV-C).
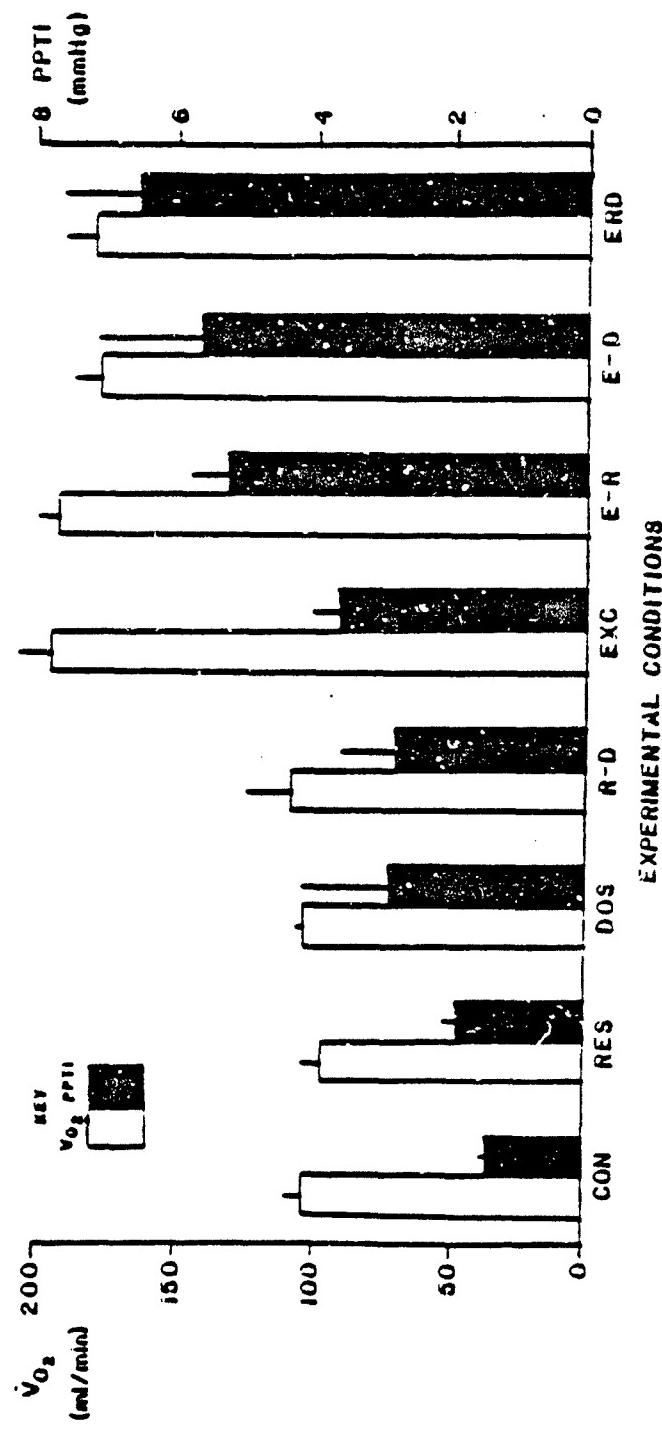
Figure IV-C. Oxygen Consumption and the Pleural Pressure

Time Index (see text for discussion).

In evaluating the time duration of the exercise bouts in
this protocol (approx. 3 minutes) one might argue that
a steady-state had not been reached and that this data
was generated during the "oxygen debt" phase of the
changing oxygen uptake (Astrand and Saltin, 1961).
However, since the resistance loading conditions of this
protocol always followed the unloaded phase without
stopping the exercise run, the data gathered during the
E*R*D condition was actually reflecting results after
a minimum of six minutes (more often 7 to 8 minutes) of
treadmill exercise. There is little doubt that a true
steady-state had been reached under these conditions of
mild exercise, yet the $\dot{V}O_2$ data obtained during this phase
of the protocol was no different from that obtained three
minutes earlier in the exercise bout without resistance
loading (refer to Table III-A and Figure IV-C). In any
lengthy, complex factorial protocol utilizing an awake
spontaneously breathing animal model, questions concerning
time-related artifacts in the data can arise, but in order
to minimize any systematic error so-induced, the challenge
conditions were held to the minimum durations that would
still render reliable results. Therefore, the conclusion is
tendered that the fall in oxygen consumption following
cholinesterase inhibition is real and requires discussion
as to plausible causative mechanisms and pathophysiological
implications.

The possible explanations for the observed fall in $\dot{V}O_2$ include: (1) redistribution of blood flow away from non-essential beds to more actively functioning muscle beds; (2) a prolongation of the time required to achieve steady-state oxygen consumption (i.e., a lenghened oxygen debt period; Astrand and Saltin, 1961); (3) a shift toward anaerobic metabolism by the exercising muscle groups (Wasserman et al, 1981).

A significant redistribution of blood from the splanchnic, renal and other vascular beds to actively exercising muscles has been shown by numerous investigators as reviewed by Rowell (1974). And it is conceivable that the oxygen consumption of those vascular beds that were drastically under-perfused might have been decreased due to shut-down of their previous metabolic levels. But the amount of oxygen made "available" in this way would be minimal. In fact others have used the assumption that oxygen consumption in these under-perfused beds does not change and have therefore calculated the amount of actual decreased perfusion that occurred by using the a-v oxygen difference across those beds via the Fick Principle (Wade and Bishop, 1962).

A prolongation of the oxygen debt period required to reach a steady-state of oxygen consumption is not an altogether unreasonable explanation of the diminished $\dot{V}O_2$; especially since two important factors that determine the time- related equilibration of $\dot{V}O_2$ with the onset of exercise have been decreased

by cholinesterase inhibition (cardiac output and minute ventilation both fell with cholinesterase inhibition during exercise). Figure IV-D depicts a schematicized diagram of the accrual of an oxygen debt from a mild exercise challenge. The dotted line depicts the theoretical extension of the rate of onset of this debt due to a diminished cardiac output and depressed pulmonary ventilation from cholinesterase inhibition. Since the blood samples were taken after 3 minutes of exercise (marked blood sample #1) in the control run (designated by the solid line curve), the calculation of oxygen consumption would be valid since steady-state (or constant $\dot{V}O_2$) had been reached. This fact is verified by blood sample #2 (after six minutes of exercise), which was no different from the one taken at 3 minutes. The same sampling procedure was accomplished for the calculation of $\dot{V}O_2$ in the cholinesterase inhibition trials (designated by the dotted line curves). If the proposed argument were accurate concerning a delayed or extended period of reaching steady-state, then we should be able to find an increase in $\dot{V}O_2$ calculated from the blood samples drawn at 6 minutes as compared with the $\dot{V}O_2$ from the 3 minutes samples. This is distinctly not the case, and thus we can dismiss this explanation as being unfounded.

Thus, we are left with the reasonable conclusion that the reduction in $\dot{V}O_2$ associated with cholinesterase inhibition
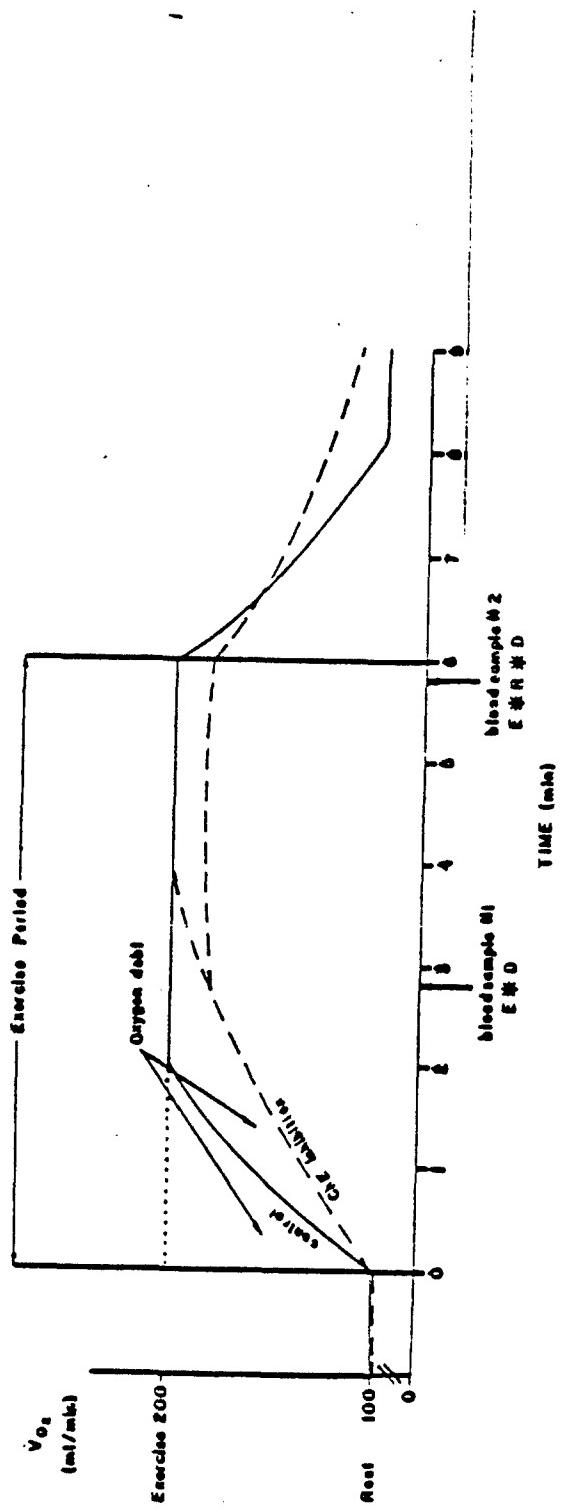
Figure IV-D. Oxygen Consumption Lag Time with the Onset of Exercise (see text for discussion).

The vertical lines delineate the onset and duration of the exercise period. The dashed line curve is the hypothetical $\dot{V}O_2$ lag curve suggested by the experimental data; it is compared to the solid line hypothetical control curve.

during exercise must be due to an increased component of anaerobic metabolism. The fact that arterial pH was significantly lowered; that arterial PCO_2 was not elevated by cholinesterase inhibition; that respiratory muscular work rate (as indexed by the PPTI) and thus total work rate was elevated (refer to Figure IV-C); and that arterial PO_2 (and thus O_2 content) was depressed; taken as a group, these findings support that conclusion. This is not to say that the whole of the exercising musculature had shifted to anaerobic metabolism; on the contrary, it seems unlikely that such a mild fixed treadmill exercise would induce such a response even under these conditions of generalized nicotinic stimulation and cholinergic depression of cardiac output. But rather, it is apparent that the combination of loads additively imposed on the respiratory musculature during this experimental protocol (i.e., a 450% increase in the muscular work of breathing from resistance loading, cholinergic broncho-constriction, and exercise-induced stimulation of ventilation) in the face of a failing cardiac output, and diminished per-fusion pressure across the inspiratory muscle beds, as well as decreased alveolar ventilation (P_aO_2 fell, while shunt fraction rose significantly after cholinesterase inhibition, especially during exercise) could have been sufficient to bring the respiratory muscles beyond the anaerobic threshold, predis-posing them to fatigue and incipient respiratory failure.

It is likely that as inspiratory resistance increases,

more metabolic energy is used to generate tension in the
muscles needed to reach the pressures required for gas flow.
Thus, a smaller fraction of metabolic work is being used
for shortening of muscle (mechanical work output). This
concept is supported by the observed fall-off in the BEI
after resistance loading and cholinesterase inhibition, as
well as a further fall in relative efficiency when the two
loads are combined (refer to Figure III-C).

Another important cardiopulmonary interaction resulting
from cholinergic resistance loads alone, or combined with
external mask resistance, merits discussion. As previously
discussed respiratory frequency and respiratory work both
increase from cholinergic stimuli while perfusion pressure
across the muscular beds has fallen, presumably limiting the
potential for increasing blood flow (perfusion) across these
strenuously working beds. But, the normal (non-cholinesterase
inhibited) response to resistive loads is to slow breathing
frequency and increase inspiratory time, whereas after neos-
tigmine we observed marked increases in frequency with no
change in duty cycle   ; but profound increases in peak
pleural pressure (PIP_{P_L}) during inspiration were observed.
The tensions that would have to be generated by the in-
spiratory muscles and the frequency at which they are driven
to generate them are undoubtedly very demanding metabolically.
But what influence does this muscle behavior have on the
myriad capillaries and small blood vessels buried deep within

the muscle mass? The answer may have been provided by
investigators in coronary physiology. Many have shown,
since Scaramucci in 1689, that the heart squeezes down
on its own blood supply; and that resistance across the
coronary beds increases proportionately with heart rate
and intramyocardial tension (Feigl, 1983), especially
in the deepest regions of the muscle mass (Archie, 1975).
There is no reason to believe that a similar mechanism
may not come into play during the high breathing frequency
and high tensions generated by the inspiratory muscles
during cholinesterase inhibition and external resistance
loading. This condition would be exacerbated by a diminished
perfusion pressure associated with a cholinergically
diminished cardiac output.

The fact that compensatory increases in ventilation
to correct the developing acidosis were strikingly absent
(in fact ventilation began to diminish after the cholinesterase
inhibition during exercise, unlike at rest where it was
stimulated) is highly suggestive of the developing inability
of the respiratory muscles to maintain appropriate levels of
performance. On the other hand, the complex inter-relationships
of autonomic cardiopulmonary control mechanisms may have been
disrupted by the cholinesterase inhibition challenge sufficient
to produce these effects. This latter hypothesis is not
refuted or supported here, but awaits further experimental testing.

Another glaringly absent compensatory mechanism
that would normally be stimulated by the blood gas changes
that followed cholinesterase inhibition was an increased
cardiac output. Mean arterial pressure fell with
the decrease in cardiac output after the ChE in-
hibition and it remained down for the entire observed
period. On the other hand, it has already been
demonstrated that the cholinesterase inhibitory effects
of neostigmine on the heart dominate any increase in
sympathetic drive to increase cardiac function. Thus
any of the above chemoreceptor or baroreceptor compen-
satory mechanisms that are vagally mediated may well be
totally disorganized by the enhanced cholinergic activity
throughout the peripheral autonomic nervous system. This
finding is totally compatible with Levy's "accentuated
antagonism" concept of sympathetic-parasympathetic in-
teractions at the heart.

D.   Summary and Conclusions

Resistance Loading. The external resistance applied
during inspiration in these experiments was selected to
approximate the range of resistances found in
industrial respiratory protective devices. With the under-
standing that dogs may respond quantitatively

differently than human subjects might, the following summary of integrated cardiopulmonary responses to inspiratory resistance loading are presented with the intent that cautious extrapolations to man might be made.

(1) Ventilation is reduced, primarily due to decreased mean inspiratory flow and inadequately prolonged inspiratory time.

(2) The muscular work of breathing, and thus the metabolic demand, of the inspiratory muscles is increased.

(3) The efficiency of the respiratory pump is decreased since it requires more energy to provide less ventilation.

(4) Cardiac work rate is slightly elevated due to a small increase in afterload to left ventricular ejection, especially during exercise; whereas cardiac output is consistently decreased at rest, but unchanged during exercise.

(5) Computed respiratory exchange ratio increases ($\dot{V}O_2$ falls while $\dot{V}CO_2$ rises), suggestive of a shift to anaerobic metabolism by the respiratory muscles.

The absolute magnitude of the above responses to resistance loading is generally greater during exercise than at rest, but the relative changes (% of control) are comparable. It is concluded, therefore, that inspiratory resistance and mild exercise are additive in their loading effects on the cardiopulmonary functional parameters studied.

Cholinesterase Inhibition. The dose of neostigmine administration in these experiments (0.025 mg/kg I.V.) was

sufficient to inhibit approximately 50% of the peripheral

cholinesterase activity as measured in whole blood;

neostigmine does not have CNS effects at this dose. This

is not considered a severe inhibition and falls within

the range of doses used therapeutically to treat myasthenia

gravis, and it is not an unreasonable amount of inhibition

to project for an agricultural or industrial exposure to

an organophosphorous insecticide (even when wearing a

"protective" respirator). The integrated cardiopulmonary

responses to this degree of peripheral cholinesterase in-

hibition are:

(1) A marked increase in the muscular work of breathing

caused primarily by cholinergic bronchoconstriction and

copious airway mucous secretions.

(2) A cholinergic reflex stimulation (probably carotid

chemoreceptor mediated) of respiratory frequency which

enhances minute ventilation at rest but is inadequate to

maintain pre-dose ventilation during exercise; This stimulated

frequency of breathing compounds the "mechanical" load effects

of the cholinergic airway resistance response to result in an

even larger energetic work rate requirement of the respiratory

muscles.

(3) The direct biochemical lesion effect of neostigmine

on the neuro-muscular junction was not studied, but based on

the consistent observation of fasciculations in other skeletal

muscles, it is presumed that an additional metabolic load

could have been incurred by the respiratory muscles by this direct pharmacologic phenomenon.

(4) A marked decrease in the effectiveness of the "respiratory pump" (i.e., less ventilation per unit of muscular work).

(5) A decreased cardiac output, secondary to enhanced cholinergic "vagal tone" on the heart, which is more pronounced during exercise (relatively and absolutely), presumably due to a parasympathetic-sympathetic interaction known as accentuated antagonism. The surprising offshoot of evaluating the hemodynamic parameters that lead to this conclusion is that through this process the heart as a muscular pump may be "protected" from excessive muscular work demands since afterload is diminished and end-diastolic ventricular volume is enlarged ($\overline{TMP}_{LA}$ rose significantly) thus placing the ventricle on a more "efficient" region of the myocardial length-tension curve. Thus,

(6) The heart as a pump is more efficient (i.e., cardiac output per unit of muscular work is increased); unfortunately the peripheral muscles, especially those of respiration, may as a result become inadequately perfused. A fuller understanding of this phenomenon may have clinical application for patients with certain types of myocardial dysfunction, for clearly neostigmine reduces the workload of the heart more than the cardiac output falls.

(7) Mean arterial pressure falls commensurate with the decreased cardiac output, and its failure to return to control levels suggests that the carotid and aortic baroreceptor reflexes may have been altered by cholinergic interference with neurotransmission at the receptors themselves, at the ganglionic (nicotinic) synapses, or perhaps even at the neuro-effector sites (Brody, 1978).

(8) Redistribution of blood volume to the lungs and heart occurs as evidenced by the elevated left atrial pressure. The amount of this blood volume shift to the lungs is difficult to quantify with the present data, but the qualitative fact has at least three important implications. First, the increased volume (and pressure in the pulmonary capacitance vessels) may change lung compliance and peripheral airways resistance; and second, the blood volume that is displaced to the chest is blood volume (and perfusion pressure) that the exercising muscle beds are being deprived of (since arterial pressure had not been maintained); third, the elevated capillary pressures may predispose to pulmonary edema and the gas exchange complications that ensure.

(9) Gas exchange in the lungs is compromised. Local areas of alveolar hypoventilation are produced, as evidenced by an increase in calculated venous admixture (Shunt Fraction).

(10) Oxygen consumption falls during exercise even though oxygen deman of the inspiratory muscles is elevated.

This suggests a shift to anaerobic metabolism of at least some working muscles. This conclusion is corroborated by a significantly lower arterial pH that appears not to be related to CO_2 content, and an elevated respiratory exchange ratio.

(11) Respiratory muscle work increased after cholinesterase inhibition, while cardiac output and oxygen consumption fell during a fixed level of exercise. The most plausible inference to be drawn is that the strenuously working respiratory muscles, which are known to be perfusion limited, have reached their anaerobic threshold and are quickly approaching a condition of fatigue. The addition of the external resistance to the exercising, inhibited dogs produced further losses in minute ventilation and durther depression of the breathing effectiveness index, while oxygen consumption did not further change and the respiratory quotient continued to rise.

It is concluded that inspiratory resistance at rest and during exercise produces mechanical loads on the inspiratory musculature and the heart, and compromises ventilatory timing and control. Furthermore, these loads are additive in nature when combined with the pulmonary mechaical changes induced by a moderate dose of cholinesterase inhibitor. However, the very additivity of thes "mechanical" loads predispose to respiratory muscle fatigue. The combined loads of resistance, cholinesterase inhibition, and exercise incrementally increase the perfusion demands of the respiratory

muscles. But perfusion capability is diminished by a
falling cardiac output, a falling perfusion pressure,
as well as compromised pulmonary gas exchange. Anaerobic
metabolism begins to develop as energy expenditure outpaces
perfusion. The respiratory muscles begin to fatigue.
Meanwhile, the heart and other exercising muscle groups
appear to be relatively unstressed . . . until ventilation
fails for lack of adequate perfusion.

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